

Strict liability in a morass

US courts seem to be bent on making a muddle over the application of the rule of no-fault insurance against faulty products. The remedy is that the issue should be taken away from the courts.

BACK in the good old days, a manufacturer in the United States would end up liable for injuries caused by his products only if the product was exceedingly dangerous or the manufacturer exceedingly stupid. The common-law standard, while perhaps not fair, was clear: unless the product was obviously defective, by failing to live up to a warranty or the manufacturer's own production norms for example, the injured party had to prove clear negligence on the manufacturer's part. In recent years, the courts have slowly but surely moved to a much tougher standard. Now, in most states, some form of "strict liability" applies. In its pure form, this doctrine holds that negligence is irrelevant: if the product causes an injury, the manufacturer pays.

In the abstract, this standard has much to commend it, however extreme it may at first appear. Strict liability distributes the cost of accidents among those who actually benefit from the sale of products — and manufacturers will inevitably pass along the cost by raising the price, in effect charging all purchasers an insurance premium. Strict liability is also arguably the most efficient means of improving product safety. The manufacturer is in the best position to reduce the total cost of accidents, choosing for itself the safety measures that will do the greatest overall good. And strict liability comes to grips with the simple reality that when someone is injured, someone has to pay; legal niceties about negligence do not change the fact that a person has suffered real harm.

In practice, liability law is a mess. One problem is the quaint notion in the United States that each of the 50 states is quasi-sovereign. The fifty supreme courts all have their own ideas on the subject of liability. In few states do statutes even determine the law, which remains largely common law or judicial precedent full of contradictions, ambiguities and, not infrequently, reversals. The fate of the victim varies greatly, depending on where he lives and where the company that made the injurious product does business.

To these geographical inconsistencies must be added the inherent logical inconsistencies in the doctrine of strict liability as it is applied. Few states have embraced the doctrine in its pure form. In general, the plaintiff must show that the product is "defective", wherein lies the rub. When the defect is the result of a kink in the manufacturing process, it is easy — you just compare the defective item with those that normally come off the assembly line. But the new rules extend the notion of defect to the design of the product. What is a design defect? A soft-drink bottle that explodes may be straightforward enough, because everyone knows you can make soft-drink bottles that do not explode, but what about a new drug, or an agricultural chemical, which may have some inherent risk?

Attempts by the various state courts to define a design defect have produced some very odd locutions. Some say a product is defective in design if it fails to be as safe as an ordinary consumer would expect it to be; others, if the mythical "reasonable person" would not have put it on the market; others still if it is "lacking any element to make it safe". These definitions appear to amount to little more than what one legal scholar calls the "I know it when I see it" rule. Manufacturers find themselves held liable for hazards they "should have known about" but did not, and even for minor side effects of drugs that were not mentioned on the warning label — even though the manufacturer is limited by law to using a warning label approved by the Food and Drug Ad-

ministration. But all this applies only in some states. And everywhere the particular inclinations of individual judges are far too influential.

The one effort to straighten out this mess is unfortunately certain to create a bigger mess. A bill now in the US Senate (S.44) would impose a uniform federal standard (a good idea) but at the cost of introducing a rash of new obfuscatory definitions. To give one example, manufacturers could raise as a defence the convention that its product is "unavoidably" dangerous. Fifty state courts will be kept busy for decades sorting out, each to its own satisfaction, what these new definitions really mean. This is why opponents of the bill have argued that the best solution is no solution, that the exquisite balance produced through centuries of evolution of common law serves justice the best. One cannot help feeling that there is a hint of professional interest here, at least from the legal scholars who manage to fill such periodicals as the *Journal of Product Liability*. The real problem with the present regime is that a disproportionate effort is required by a plaintiff in travelling through arcane legal byways. The result is inconsistency and, not incidentally, big legal fees.

True federal reform should bite the bullet and take victim compensation out of the courts. The analogy is workers' compensation — a system that has worked well. Employees injured on the job can no longer sue, except in extreme circumstances; in return, their employers agree to compensate injuries on a no-fault administrative procedure. If true strict liability is the standard, there is simply no justification for judicial proceedings at all. The federal government should be aiming at a simple procedure based on the principle that manufacturers are responsible for the damage that their products cause, and under which the only question to be answered is the equitable settlement of compensation.

Only three kinds of exceptions would be necessary. The first, again by analogy with workers' compensation, is that in cases of gross negligence or recklessness on a manufacturer's part, plaintiffs would be allowed to sue in the courts — but would have to meet the strict standards required for the award of punitive damages. Punitive damages should also be allowed for cases in which a manufacturer has failed to provide a proper warning when it clearly knew of the danger, for otherwise the manufacturer would find little incentive under a no-fault compensation scheme for full disclosure. And it would be necessary to legislate for cases in which a manufacturer selling a defective product has gone out of business before damage has been caused — a set of circumstances not catered for under present law. □

Patrons for universities

The UK Government should help its universities find patrons and be a better patron itself.

IF portents can be relied upon, the relationship between British universities and the government which supports them is in a state of modest flux. But as things are, there is no way of telling what changes lie ahead. Much will hang on how seriously the British Government responds to the deep anxiety about the state of the research enterprise in Britain (see *Nature* 26 July, p. 261). But there are more particular developments ahead that will call for



some kind of a response. Next month, for example, the University Grants Committee will deliver its promised summary of replies to the questionnaire put out last year to all British universities. Most probably, the committee will be found to have toned down some of the more vigorous defences of established practices by its constituents but to have made up for defaults of this kind by throwing its weight behind the general complaint that the university system is indeed in danger. Thus the committee is unlikely to endorse the extreme demand that academic tenure is inalienable, but will stick up to the best of its ability for the notion that universities should be free to govern themselves. That, at least must be the hope.

Straws in the wind of change are apparent in two other aspects of the relationship between government and the universities — finance (which perforce comes from the government in present circumstances) and objectives (where the two parties need to reach an understanding). Since the beginning of the present round of money troubles (in 1981), budgets have been made to shrink further than has been necessary on demographic grounds. No doubt the next forecast of public expenditure will show a cautious retreat from these past privations. But the universities should not set too much store by that. The important need is that the structure of the financial relationship between the two partners should be redesigned.

A study by the Committee of Vice-Chancellors and Principals last month shows the urgent need for change. For British universities collectively, direct subventions by the government accounted for roughly a third of total income in the mid-1930s, but now the proportion has doubled. At the same time, government indirectly pays for roughly a quarter of the cost of running British universities, partly by its payment of university fees and student maintenance grants, partly through the research councils. During this traumatic half century, meanwhile, the total income of British universities from endowments and donations has shrunk from more than 17 per cent to a mere 1 per cent of the total. Put simply, the university system has grown much more quickly than its private sources of support. Now, while some institutions (chiefly Oxford and Cambridge) enjoy an enviable independence from government, most institutions are firmly in its pocket.

The vice-chancellors' committee has some sensible if modest remedies to suggest. British universities have been feckless in failing to draw on the support of their alumni, a well-established practice in the United States, and should mend their ways. And the British Government, which is forever urging the universities to forge closer links with industry, should revise the regulations on the taxation of corporate benefactions so as to ensure that gifts are not a charge on profits after tax but on general income. Most probably the committee, under Dr John Burnett of the University of Edinburgh, goes further than it knows the government will countenance when, with an envious glance at Alberta, it suggests that the government should be prepared to match, pound for pound, what the universities are able to raise from potential benefactors. But the principle that even the government should in the long run benefit if the universities are helped to a greater degree of independence is beyond dispute. The government should promptly say yes.

Two other financial issues are more politically charged. A large part of the reason why the universities are saddled with iniquitous quotas on student numbers is that there is no mechanism by which the cost of student maintenance can be controlled under present law. The committee moves further than any comparable body in recognizing that Britain is unique in its obligation that the cost not merely of students' fees but of their maintenance will be carried by taxation (unless the parents are well-heeled). Switching to some other system is urgently necessary. The committee flirts with schemes for student loans (which will not save money in the short run), and indeed some reform along these lines should be attempted. But the best course for Britain would be a return to the old system of scholarships for able young people with no artificial restrictions on student numbers. The question whether British universities should be compelled (as now) to charge "economic

fees" to students from overseas should be reopened.

What is all this for? The British Government now finds itself in the curious position of being accused of running its universities on too short a shoestring while asking that their contribution to the national economy should be more direct and more valuable. The latest evidence to this effect is a report from the Department of Industry, published at the end of last month, which acknowledges the likely shortage of trained people in the technical fields in which it is especially anxious to succeed — those related to information technology. Mr John Butcher, the minister at the Department of Industry who was chairman of the committee responsible for the study, seems to have been seized with the importance of the objective — so much so that there is once again talk among government officials of organizing a "switch" from arts and humanities to engineering. Again, the next few months should show whether the government will put its money where its mouth is. There is no other way of getting what it wants. □

Minds at the helm

Moves to screen US operators of nuclear power plants smack of pseudoscience.

THE US Nuclear Regulatory Commission (NRC), which professes to be concerned about the mental stability of nuclear power plant employees, is itself behaving rather erratically. It is now proposing regulations that would require "psychological screening" of applicants for jobs that allow "unescorted access" to critical areas of power plants; once on the job such employees would be subject to "continual behavioral observation" by supervisors.

The NRC staff has diligently produced a page of fine print to guide supervisors in this task. The "behavioral changes" they will be looking out for include excessive doodling, lack of a sense of humour, poor eye contact, impatience, borrowing money, yawning, complaining of back pain and playing pranks. (The last point is particularly interesting as, according to the guide, another warning sign of a worker's psychological impurity is that *other* workers play pranks on *him*. Apparently everyone is suspect when pranks are involved.).

After detecting these or any of more than 100 other suspicious signs, the supervisor will refer the suspect back to the access authorization office — apparently the same people who administered the "personality test" on him when he applied for the job — for an expert evaluation. There, judgement will be rendered with the assistance of trained professionals, psychiatrists and psychologists who, with scientific precision and dispassion, will determine whether the employee will go mad at the control panel one day.

Unfortunately, many psychiatrists and psychologists (more of the former) seriously believe that they can predict a person's future behaviour. That view has been accepted by more than a few states, which have in the name of science and reason turned over to these professions the authority to order the release of criminals committed to psychiatric institutions following verdicts of not guilty by reason of insanity. Their ability to forecast the behaviour of these subjects is unimpressive. The misplacement of judicial responsibility is enormous. NRC is now asking for comments on its proposal; it is probable that psychiatrists and psychologists will like it if no one else will.

Employers of all sorts, of course — not just nuclear power plant operators — are known to resort to all kinds of strange methods to find the perfectly trustworthy employee; thus the popularity of lie detector tests, handwriting analyses and asking an unsuspecting job applicant to open a window that is nailed shut. None is scientific, all are capricious denials of an employee's reasonable expectation to be judged on his merits and his performance. What NRC is proposing is no more scientific and no less capricious. It will substitute undefined and ultimately pseudoscientific criteria for objective rules of employee conduct and performance; it will elevate a pseudoscientific judgement above the judgement which a supervisor truly can make: the important question is whether the employee doing his job well. □

US nuclear power

Silver lining shows for industry as demand grows

Washington

ALTHOUGH 1984 is not the best of years for the US nuclear power industry, industry spokesmen maintain that neither is it the worst, and point to several hopeful omens.

The most recent and tangible success was the decision on 2 August by the Nuclear Regulatory Commission (NRC) to approve a licence application from the Pacific Gas and Electric Company to carry out full-power testing of its Diablo Canyon-1 plant in California. The utility discovered in September 1981 that modifications to the original design made necessary by the discovery of a nearby geological fault had been carried out incorrectly, apparently because the wrong plans had been used. Now, with the errors corrected, Pacific Gas hopes to be supplying electricity to consumers as early as October if tests go according to plan.

The industry has also taken heart from the recent decision to resume construction at the Seabrook plant in New Hampshire. With the plant 85 per cent completed, construction had been halted, because of escalating costs. According to the Atomic Industrial Forum (AIF), the will to put together a financial rescue package was mustered after it became clear that without Seabrook, New Hampshire's electricity would have to be supplied by a nuclear plant over the border in Canada.

Another encouraging sign for the industry is the sudden rise in electricity demand. After years of little or no growth, demand in the United States jumped by 8 per cent according to the latest annual figures. AIF predicts that existing excess generating capacity will be quickly depleted if this growth continues.

The immediate picture still has its bleak spots, however. So far this year, 13 construction projects have been delayed and four have been cancelled; five operating licences have been issued and three reactors have started commercial operations. And still no new orders have been placed for nuclear plants since 1978.

The spectacular cost overruns that have attended the recent cancellations have not helped. The most recent casualties, the Midland 1 and 2 plants in Michigan, were cancelled last month after \$4,000 million had been spent. The plants' owner, the Consumer Power Company, says the decision to cancel was taken after the breakdown of negotiations with consumer groups over how rising costs could be met. The company had offered to absorb \$1,000 million dollars of the cost and the two sides were not very far apart when it became clear that Wall Street would not bail out the project. The company has applied for com-

pensation for the construction costs, saying that the plant will be left in a state that would make it possible for a future owner to complete the construction work, and is now trying to avoid bankruptcy. And in New York, a similar fate may await the Long Island Lighting Company, due to technical problems and delays at its Shoreham plant and the unwillingness of the local authorities to cooperate in emergency planning.

Financial woes are casting a cloud even over the industry's recent success stories. The financial consequences of the earlier blunder at Diablo Canyon are still hanging over the utility; it will be up to the California Public Utilities Commission to decide how much of the \$5,100 million construction costs can be passed on to consumers.

On a different front, the industry may benefit from the expected publication this year of new, lower "source terms" for nuclear plants by the Nuclear Regulatory Commission. The commission believes

that the present source terms, which define the likely release of radioisotopes into the environment during an accident, are unnecessarily cautious.

One immediate consequence of lowered source terms would be that emergency planning requirements could be relaxed. But any such proposal would be certain to meet stiff opposition: the Union of Concerned Scientists (UCS) has already declared its belief that source terms used at present are too low, rather than too high.

Another possible effect of revised source terms envisaged by UCS would be to alter assessment of the maximum liability of a plant operator in the event of an accident. The liabilities of nuclear operators are at present covered by the Price-Anderson insurance plan, which puts a ceiling on liability and includes provisions for costs to be shared out between nuclear operators in the event of a serious accident. Price-Anderson expires in 1987 and in the next Congress discussions will start in earnest over how the scheme should be renewed. One set of proposals already aired by the Nuclear Regulatory Commission would have the effect of substantially increasing operator liability, a suggestion the industry is rather cool about. But UCS believes that new source terms would be used to argue for a lower operator liability, rather than a higher one.

Tim Beardsley

Biotechnology centres

Spanish self-consolation

Barcelona

THE Spanish Government is to set up a National Centre for Genetic Engineering and Biotechnology in Madrid.

The idea arose last year before the Madrid meeting of the committee of the United Nations Industrial Development Organization (UNIDO) on the foundation and location of an international biotechnology centre. Officials from different ministries agreed to contribute funds from their respective budgets to support Spain's candidacy, but when the UNIDO committee decided to split the international centre between India and Italy, the commitment of the Spanish authorities to create a biotechnology centre in Madrid persisted.

A committee including government officials, industrialists and scientists has been at work for the past year, and a planning document has been approved and released. The committee has also approved the creation of a Centre for Microelectronics, to be split between Barcelona and Madrid.

The National Centre for Genetic Engineering and Biotechnology will have a staff of 220-260, including about 40 scientists. The initial group of scientists will come from laboratories already working in related areas, mainly in institutes of the Consejo Superior de Investigaciones Científicas (CSIC, the Spanish science

research council). Because of the lack of experience in some of the fields covered, other scientists will be recruited from elsewhere. It is likely that this and related projects will absorb many of the new positions to be created in CSIC.

The new centre will be housed in a 10,000m² building on the campus of the Autonomous University of Madrid, not far from the Centre for Molecular Biology, one of the leading Spanish research centres. The lines of research to be followed at the new centre will include molecular genetics, immunology, plant molecular biology, biochemical engineering and industrial microbiology. The total cost of the project is estimated at about 3,000 million pesetas (US\$20 million) over the next three years.

The creation of the biotechnology centre is one outcome of the government's plan to concentrate research spending on a few priority fields. The new centre will absorb more than 70 per cent of the money allocated to the government's outline plan for the development of biotechnology. At the same time, the funds available to other projects have been either maintained at previous levels or reduced. For example, the funds available this year for molecular and cellular biology and biochemistry from the main funding agency represent about a third of those awarded to the new centre.

Pedro Puigdomenech

Star wars

Strategic weaknesses made plain

Washington

PRESIDENT Reagan's proposed "Star Wars" defence is technically unfeasible and strategically unsound, according to a new study headed by Sidney Drell, a physicist at Stanford University. The study* released last week, recommends that support for the so-called Strategic Defence Initiative be limited to the present level — roughly \$1,500 million per year — and that the programme be restricted to basic research that would serve primarily as a "hedge" against possible Soviet advances.

The study warns that the very act of intensifying a research and development programme in this area could be strategically destabilizing: "Even if a deployed system never materializes, increased instability can result as both sides build up their forces to preserve their deterrent capabilities . . . to match each other's anticipated ABM capability."

The administration has requested \$1,777 million for fiscal year 1985, and wants to spend a total of \$26,000 million over the next five years. The House of Representatives cut the 1985 request by \$400 million, the Senate by \$150 million but the entire defence authorization bill is now deadlocked in a conference between the two in which the most contentious issue is the procurement of MX missiles.

The Drell study treads the increasingly familiar ground of technical objections to an effective space-based defence, but also levels a series of criticisms based on the effects of such a defence on nuclear deterrence and on stability in times of crisis. The administration, in the face of technical criticisms of the notion of an air-tight defence — one that could render nuclear weapons "impotent and obsolete" in President Reagan's words — has recently begun to suggest that even a leaky defence would enhance stability. In a recent briefing for foreign correspondents, Under Secretary of Defense Fred Ikle said that a "partial" defence against Inter-Continental Ballistic Missiles (ICBMs) could "protect retaliatory capabilities . . . and thus really introduce additional stability". The reasoning is that even a rudimentary defence would introduce so much uncertainty into Soviet strategic calculations that they would not risk a first strike — success would be too uncertain. But the Drell study claims that an effective but imperfect defence would diminish not only the incentive for a first strike but also the reliability of the Soviets' retaliatory capability. If both sides have such a defence, during a crisis "each could fear that the other would calculate his situation to be better (however bad) if he struck first" — knowing that his defence would have to deal only with a crippled retaliatory force.

The study argues that there are "surer

and less risky ways to enhance our deterrent" than deployment of a massive Anti-Ballistic Missile (ABM) system; among these are the recommendations last year of the Scowcroft Commission to replace multiple warhead land-based ICBMs with single-warhead, possibly mobile, "Midgetmen" missiles — a less easy target for a Soviet strike. Drell notes that the ABM Treaty itself has enhanced the US deterrent: an ABM would protect only ICBMs, but the treaty, by assuring successful penetration of a retaliatory attack, enhances the deterrent value and of ICBMs and submarine-launched missiles.

Drell's most telling objection is perhaps that the Soviets are unlikely to stand still in the face of a US deployment of a space-based defence. The development by the United States of multiple warhead missiles in the late 1960s was in fact a direct response to observed Soviet ABM activities. Steps that the Soviets might take in response to a US space-based ABM include countermeasures (hardening of missiles against lasers and particle beams); an expanded ICBM force (Soviet SS-18 missiles can carry up to 30 reentry vehicles, although they are now limited to 10 by SALT II, so it would not be difficult for the Soviets quickly to boost the number of warheads or decoys it could send up); a shift to long-range cruise missiles, which can evade a space-based ABM; and antisatellite weapons to destroy a US space

defence. (As the report by the Office of Technology Assessment earlier this year noted, the "pop-up" defence advocated by Edward Teller as a solution to the inherent vulnerability of a space-based system would require an instantaneous launch in response to detection of a Soviet attack, and would have to be propelled by a booster thousands of times larger than the Saturn V in order to get into position quickly enough. Even then, small changes in Soviet ICBM characteristics — a few seconds' shorter burn time for its boosters, for example — would mean that the defence could not get up in time.)

The Drell study and the administration agree that US deployment of a space-based defence would entail abrogation of the ABM Treaty. Even testing might. Article V forbids the parties to the treaty to "develop, test or deploy ABM systems or components which are sea-based, air-based, space-based or mobile land-based". And "Agreed Statement D" of the treaty seems to suggest that exotic ABM systems (not involving missile interceptors) not foreseen when the treaty was written could be developed, tested or deployed only by amendment of the treaty. Drell argues that, besides protecting US retaliatory capability, the ABM Treaty is the "clearest demonstration that escape from total political and strategic confrontation is possible and can have practical effect".

Stephen Budiansky

**The Reagan Strategic Defense Initiative: A Technical, Political, and Arms Control Assessment, Sidney D. Drell, Philip J. Farley and David Holloway, Center for International Security and Arms Control, Stanford University.*

Academic tenure

British rights to go

BRITISH universities have washed their hands of the government's plan to legislate to extinguish academic tenure. The chairman of the Committee of Vice-Chancellors and Principals, Lord Flowers, has now told the government (in a letter published two weeks ago) that he cannot deliver the agreement of the universities voluntarily to change the rules on tenure and that, in the circumstances, "it now remains for you to decide what you want to do". Sir Keith Joseph, Secretary of State for Education and Science, told the House of Commons on 1 August that the government will therefore go ahead with plans to extinguish tenure.

The prospects for speedy change are only slim. The government takes the view that because the complicated legislation that will be required to set up parliamentary commissioners with powers to alter university statutes could not be ready for the parliamentary session beginning in November, powers to appoint commissioners would not be granted until mid-1986 at the earliest. That could mean that the first enforced changes of university statutes, which will not affect the rights of

academics now in post, can be expected only at the end of the decade.

Views among academics on the latest development in this long-standing dispute are sharply different. Some think it a pity that Lord Flowers' letter to Sir Keith Joseph did not take the opportunity to reiterate ingredients of academic tenure in whose defence vice-chancellors would be prepared to go to the stake. Ms Diana Warwick, general secretary of the Association of University Teachers, says that the letter shows that the universities have handed the problem back to the government "with ill-concealed relief".

Vice-chancellors, on the other hand, set great store by the understanding that has apparently been reached with Sir Keith that there will be continuing consultation about the drafting of the impending legislation, and some even claim to have detected signs that Sir Keith is no longer convinced that changing the pattern of academic tenure is more than a symbolic gesture. They also guess that many universities will, under the threat of legislation, change tenure rules off their own bats without waiting for government intervention. **John Maddox**

Nuclear shipping

Japan's vessel still adrift

Tokyo

THE Japanese Government has decided to provide yet more funds for the ill-starred nuclear ship *Mutsu*, in spite of a wealth of scientific evidence for its own advisory boards that the project will serve no further useful purpose. The decision is partly a compromise between the interests of different scientific agencies and partly a result of the bureaucratic fallacy that "We must spend more or the money we have already spent may come to nothing".

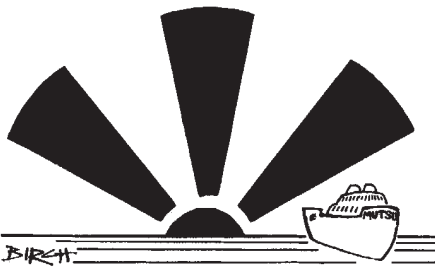
The saga of the *Mutsu*, wandering Japan's seas unable to find a home, unwanted and gobbling up vast quantities of public money, has long verged upon farce. After its launch in 1968, the 8,200-ton ship was fitted out in Mutsu City and was ready for sea trials by 1972. But the *Mutsu*'s builders had reckoned without the local fishermen, who were terrified that radioactive discharges could ruin the local scallop fisheries upon which they depended for a living. Only two years later, when a typhoon had dispersed a blockade of 250 small boats, was the *Mutsu* able to break out of the harbour — at midnight, appropriately enough.

A few days later, the reactor went critical — and then virtually everything the ship's opponents feared would happen did happen. The reactor began to leak: not a great deal, but as the ship was now 500 miles from shore, the crew had to improvise repairs with whatever was at hand. The ship's cook seems to have played a vital part in affairs, for rice was boiled up with borates in order to add to the radiation shielding; when this proved only a partial success, cast-off socks were added to the mixture. It was now essential to get the ship back to port for repairs. But having been rid of the *Mutsu*, the local fishermen were adamant that it should not return. For a month and a half, the ship drifted offshore while the mayor negotiated with the government. Victory for the locals was finally complete: the ship was allowed back only on condition that it left permanently within six months, that all shore-based facilities for the ship were removed and that a programme of public works would be paid for by the government along with compensation for any fall in sales of the local shellfish caused by the bad reputation the ship had given the area.

From Mutsu City in the far north of Japan, the *Mutsu* set off for the south, meeting flotillas of protesters wherever it went. A few years were spent in Sasebo, Nagasaki, where new radiation shielding was added and then the ship had to move on again, this time back to the north, to Ominato in Aomori prefecture. By 1984, 57,000 million yen (£175 million) had been spent — almost ten times the original cost of the ship — on repairs and more repairs and on liberal handouts to mollify local

protesters. More than ¥12,000 million was spent on repairs at Sasebo alone without it being clear whether they were complete. In the past two years, the ship has made one voyage — winching itself forward 100 metres to check an anchor. Even this move required negotiations with two ministries and two local authorities.

With this background and the knowledge that a further ¥100,000 million



would be required to bring the ship into full service, it is hardly surprising that the ruling Liberal Democratic party's Science and Technology Committee recommended this year that the *Mutsu* be scrapped. It claimed that by the time further work was complete, the ship would be out of date and that, in any case, the era of nuclear ships the *Mutsu* was meant to herald was showing no signs of arriving. But while the report was being considered, the government decided that a permanent home for the ship could be found only by building a

new ¥60,000 million port. Construction began a few months ago at Sekinehama, close to Mutsu City, even though no decision on the future of the ship has been made.

The government was not able, however, to make the decision to scrap the ship. Once again, it decided to provide the lowest level of support needed to keep its experiment alive. One factor which seems to have swayed the government, besides the vested interests of the Science Technology Agency which has been responsible for the *Mutsu*, was the negative reception of the mission which visited the United States and Europe in the hopes of gaining cooperation on nuclear ship developments. Nuclear-powered ships are seen in the West as primarily of military importance and no country seems to have been prepared to share its secrets with Japan. Even if the prospects for commercial nuclear ships are poor, those seeking further support for the ship made much of the view that whatever Japan is to learn about nuclear ships, it will have to learn from the *Mutsu*, outdated or not.

Commercial nuclear ships, first advocated in the 1950s, have been the victims of economics, chiefly the high capital cost of the nuclear reactors necessary for seaboard operation and the shore installations needed to maintain them. A US vessel built in the 1950s has long since been abandoned. The Soviet Union operates a nuclear ice-breaker, but for the rest, the only nuclear ships at sea are military submarines and aircraft carriers.

Alun Anderson

Japan to import US idea

Tokyo

FOREIGN governments have long envied the efficiency of Japan's Ministry of International Trade and Industry (MITI), wondering whether they should have something similar — France has even gone so far as to set up an imitation ministry. But Japanese Government officials are just now wondering whether they should be imitating the United States, setting up an organization modelled on the Office of Technology Assessment.

The principal cause of this Japanese soul-searching is the money thrown away on the nuclear ship *Mutsu* (see accompanying article). Why was the project allowed to drag on year after year without any clear goal in sight? The answer seems to be the lack of overall control of, and coordination between, the different ministries and agencies concerned with science and technology.

The Science and Technology Agency (STA), which was responsible for the development of the ship, has been determined to keep on extracting funds for it at any price. Other scientific groups have long favoured scrapping the ship. The result has been compromise — the *Mutsu* has spent

the past ten years being repaired, instead of being either scrapped or made to produce some tangible scientific result.

This is only one example of the unprofitable disagreements between ministries and the lack of a powerful central review body. Others are the competition between the Ministry of Posts and Telecommunications and MITI over electronic technologies (see *Nature* 308, 763; 1984) between the Ministry of Education, Science and Culture (MESC) and MITI over software copyright (see *Nature* 309, 393; 1984) and between MESC and STA over space development, each maintaining its own independent rocket launcher research programme (see *Nature* 305, 374; 1983).

Now the director-general of STA, Michiyuki Isurugi, has revealed plans for a new scientific review body apparently modelled on the US Office of Technology Assessment, which has been praised in Japan for aiding the suspension of supersonic passenger plane development and for opposing increased spending on fast breeder reactors. The final shape of the new body is expected to be decided in the autumn.

Alun Anderson

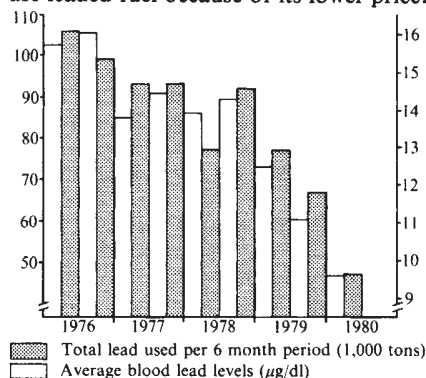
Gasoline lead

Ruckelshaus makes a move

Washington

CITING "overwhelming" evidence that lead in gasoline is a threat to human health, the Environmental Protection Agency (EPA) has proposed cutting back the lead content of gasoline by 91 per cent.

Besides bringing about a direct reduction in the amount of lead going into the atmosphere, the new rule, if adopted, is expected to increase the price of leaded gasoline at least to that of unleaded, thus discouraging the practice of fuel switching. An estimated 13 per cent of car owners equipped with catalytic converters illegally use leaded fuel because of its lower price.



The leaded fuel then destroys the catalyst, causing another pollution problem: hydrocarbon and carbon monoxide emissions increase by at least a factor of four.

The Reagan Administration had originally proposed doing away with, or at least relaxing, the old lead-in-gasoline standard of 0.5 grams of lead per gallon applicable to the average lead content of all gasoline, leaded and unleaded. After complaints from health experts, the administration changed its mind and, in the autumn of 1982, imposed a standard of 1.1 grams of lead per gallon of leaded gasoline, which EPA reckoned was equivalent to 0.5 grams per average gallon standard, and which would reduce lead even more as the use of leaded gasoline declined.

Last week, EPA Administrator William Ruckelshaus acknowledged that the reduction had not occurred as quickly as had been expected, largely as a result of fuel switching. The agency had predicted in 1982 that lead usage would be 21.4×10^9 grams by 1988 but its revised estimate is 35.7×10^9 grams, up by 67 per cent.

The new standard, 0.1 grams of lead per gallon of leaded gasoline, is the minimum amount needed for older engines that require lead as a valve lubricant. EPA estimates that by the mid-1990s, all of these older cars will have been retired; at that time, an absolute ban on lead in gasoline may be proposed.

EPA is considering two options for introducing the new standard. The first would impose it in 1986, the second would phase it in, reaching 0.1 grams per gallon by

1988. There will be hearings on the proposal at the end of the month.

The lead proposal is one of the very few that the Reagan Administration has made to tighten environmental regulations. Ruckelshaus last week cited the persuasive evidence linking lead in gasoline and lead in children's blood. His case was probably not hurt, either, by a cost-benefit analysis showing that the cost of the new regulation, \$375 million, is vastly exceeded by reduced medical costs and reduced vehicle maintenance costs of \$1.8 million. The Office of Management and Budget, the well-known scourge of environmental regulation, could not argue with those numbers.

Stephen Budiansky

Biophysics congress

Stay-away hosts

THE eighth international biophysics congress held at Bristol (United Kingdom), from 29 July to 4 August and organized by the International Union for Pure and Applied Biophysics (IUPAB) and the Royal Society, attracted approximately 1,300 people. The triennial conference aims both to provide a forum for discussing latest developments in a particular field and to give people a flavour of the biophysical world outside their own part of it.

A vast range of topics was considered at Bristol and the small number of invited speakers in each session were provided with enough time to present their own work in relation to a general framework. The central emphasis at this conference was on atomic interactions, structural transitions and dynamics of macromolecules and membranes. Recent biophysical advances in resolving higher levels of molecular organization (motile and structural proteins, sensory receptors) were described with the use of computer graphic, nuclear magnetic resonance (NMR) and image reconstruction methods. Two practical applications of biophysics were much in evidence, *in vivo* NMR, the non-invasive technique with huge medical potential, and the manufacture, processing and storage of foodstuffs.

International enthusiasm for the conference (over 350 delegates from the United States alone, good representations from the Soviet Union and China) was not mirrored by the UK attendance, which was only one-sixth of the total. Clearly many "soft money" scientists were not prepared to pay for the cost of the meeting from their grants. However, a successful conference was achieved by almost 1,000 poster displays of current research, efficient organization and pleasing surroundings in Bristol's floating harbour. Sightings are already set towards Jerusalem in 1987.

Maxine Clarke

Sakharovs

More anxiety in West

SCIENTISTS from 13 countries, including several Nobel prizewinners, will this week launch a new action in support of Dr Andrei Sakharov and his wife, Elena Bonner. Those concerned will offer to maintain a continuous presence in Moscow of volunteer "good faith witnesses" (on a rotating basis), if the Soviet authorities will allow Mrs Bonner to travel abroad for ophthalmic treatment.

It was in protest at the refusal of the authorities to permit such treatment that Dr Sakharov began his reported fast in May. The Soviet official line, however, is that Mrs Bonner can receive adequate treatment in the Soviet Union and that she wishes to go abroad only to carry out anti-Soviet propaganda.

Those who have agreed to act as "good faith witnesses" are treating the commitment seriously — to the extent that several of them have carefully specified the dates when they have unbreakable professional commitments. Under the circumstances, however, the likelihood that their offer would be accepted seems extremely remote — leaving aside the question of what reprisals the Soviet authorities could take on the "witnesses" should Mrs Bonner either fail to return or give press interviews which the Soviets might consider had a political content.

Moreover, according to sources in Moscow, Mrs Bonner has succeeded in getting a letter to her friends there, for the first time since May, in which she says that she expects to be put on trial before the end of the month, on a charge of anti-Soviet slander. Dr Sakharov's relatives abroad are with good reason cautious about any news purporting to come from either of them, but his step-daughter, Mrs Tania Yankelevich says that the family are inclined to accept this particular letter as authentic.

The relatives, however, still have no reliable news about Dr Sakharov. Mrs Yankelevich, says, however, that the family are treating seriously reports that, in June, a team of experts on forcible feeding was sent to Gor'kii, and also the report in mid-July that Dr Sakharov had been given injections of psychotropic drugs. Even here, however, there is some lack of clarity, since the psychiatrist said to be commuting to Gor'kii by special plane, Dr Vladimir Rozhnev, is a specialist in psychotherapy and hypnosis not in drug-treatment. It is possible, of course, that a two-pronged course of "treatment" has been used, particularly since (according to Mrs Yankelevich), during Sakharov's previous protest fast, in November 1981 (to obtain an exit visa for his stepson's fiancée) "psychotherapeutic" pressure (but no drugs) was used in a vain attempt to get him to abandon his fast.

Vera Rich

Artificial fertilization

US Congress plans to act

Washington

A NATIONAL commission to monitor the use of *in vitro* fertilization and related techniques in the United States moved a little closer last week. A House of Representatives subcommittee heard arguments for guidelines governing the practice from a wide range of legal, medical and ethical authorities, and the subcommittee's chairman, Representative Albert Gore (Democrat, Tennessee), has indicated that he will push for such a commission to be established under the National Institutes of Health (NIH) authorization bill.

Many of the witnesses at last week's hearings, held by the oversight and investigations subcommittee of the House Committee on Science and Technology, drew attention to the present chaotic state of laws impinging on *in vitro* fertilization (IVF). As IVF techniques are new enough to be considered still experimental, there could be legal challenges to some aspects of IVF in almost half of the states. Many states also have (inconsistent) laws that could affect embryo transfer, embryo freezing or commercial transactions involving gametes or embryos.

The House of Representatives NIH authorization bill, sponsored by Gore, includes a provision to establish a presidential commission on human genetic engineering. The Senate version of the NIH bill includes instead a provision to authorize Congress's Office of Technology Assessment to carry out studies in bioethics. Gore now believes that the scope of the proposed genetic engineering commission should be broadened to include matters related to new reproductive technologies, because the questions raised by the techniques are likely to be similar. This change of heart will make it easier for a compromise with the Senate version to be reached.

Aides are however anxious to point out that there remain substantial differences between the two versions. The House wants to see a very "visible" commission that would have lay and scientific representation, hold public meetings and publish annual reports. The Senate proposal for bioethics studies, in contrast, would leave the work very much under congressional control.

The two days of public hearings last week were enlivened by the unexpected appearance of a group of mothers of children conceived through *in vitro* fertilization, some accompanied by their offspring. One of the mothers, Mrs Barbara Brooks, supported the calls for a presidential commission, arguing that it would in time make IVF techniques more widely available.

Expert witnesses were almost unanimous in believing that the United States has

fallen behind in its thinking on IVF. A report on the subject prepared in 1979 by the Ethics Advisory Board of the Department of Health and Human Services has been left to gather dust, while the board itself has been disbanded. Federal funds cannot now be used to support research involving IVF because research proposals have to be approved by the board to qualify for support. Researchers appearing before Gore's subcommittee last week argued strongly that a mechanism to allow federal funds to be used in IVF research should be reinstated.

None of the witnesses called last week argued against IVF itself, although many had reservations about the related freezing of embryos for future implantation or research. The questions of the rights that attach to an embryo, and whether an embryo can be owned, were seen by many as central to the debate. At one extreme,

Professor John Robertson of the University of Texas argued that any restrictions on commercial transactions involving embryos or gametes could violate what he saw as the constitutional rights of a couple to reproduce. At this Representative Gore appeared momentarily to forget his role as investigator, and started arguing the point.

On Thursday, attention turned to the technique for embryo transfer known as lavage, in which a fertilized egg is transferred from the uterus of a donor mother to the recipient. The practice was discouraged by the Warnock committee but is being practised in the United States by Professor John Buster, professor of obstetrics and gynaecology at Harbor UCLA Medical Center. The main controversy with lavage is however because Buster's commercial sponsors have applied for patent protection not only for the equipment and materials used, but also for the lavage procedure itself. A number of questions were asked last week about the propriety of patenting a reproductive technique.

Tim Beardsley

US plutonium

Japan enjoys inside track

Washington

THE Reagan Administration has approved the transfer of 189 kilograms of plutonium from France to Japan, ignoring protests from 15 senators and congressmen that the proposed shipment by sea is too dangerous and sets a bad precedent.

The plutonium, in the form of plutonium oxide, was reprocessed in France from spent fuel earlier shipped from Japanese reactors. The material is subject to US control because it is derived from uranium supplied to Japan by the United States.

The transfer is the first to be approved since the enactment of the Nuclear Non-proliferation Act in 1978. The act lays down strict conditions on all nuclear transactions between the United States and other countries; in the case of so-called "retransfers" of nuclear material originally supplied by the United States, the Secretary of Energy must determine that it is provided with adequate physical security and safeguards and that it is not a proliferation risk. The secretary's decision is not subject to congressional approval in the case of retransfers.

The United States has already approved the reprocessing of spent fuel containing 14,000 kg of plutonium, so the present Japanese request is expected to be just the first of many.

Senator William Proxmire (Democrat, Wisconsin) and Representative William Ottinger (Democrat, New York), who led the protest, said they were particularly concerned that it had been approved without consideration of Japan's actual need for the fuel. According to an analysis prepared by the Federation of American Scientists

(FAS), Japan has so far obtained 2,540 kg of separated plutonium, half from its own reprocessing plant at Tokai-Mura and half from Sellafield in Britain. FAS estimates that at most 1,630 kg of that has been placed into Japan's experimental breeder facilities and its prototype low-enrichment plutonium reactor (Fugen, a plant of roughly 160 MW), leaving at least 900 kg, a four-year supply for current programmes. Proxmire said that it was a dangerous precedent not to consider need, a situation that could lead to the accumulation of large reserves of unneeded plutonium.

The approved shipment contains enough plutonium to produce about 30 nuclear weapons. Japan has already contracted with France and Britain for the reprocessing of spent fuel containing 35,000 kg of plutonium, according to David Albright of FAS; that would mean 140 shipments of the size of the present one, at a rate of roughly one per month.

Another concern of Proxmire and the others is the safety of sea shipment. The US military is to escort the cargo vessel "in designated areas", according to the Department of Energy; further details, including the shipping date, are classified. Proxmire said that a level of protection at least equal to that accorded nuclear weapons should be provided instead.

Proxmire and the other legislators aired their concern in a letter to President Reagan. Last week, they received an unusual rebuff when the White House Deputy Press Secretary, Larry Speakes, speaking from California where the President is on vacation, said the President would not consider the issues that they had raised.

Stephen Budiansky

French research

Agenda for a new minister

THE new French Prime Minister, 37-year-old Laurent Fabius, ex-minister of industry and research, will help to protect research from the rigours of a difficult budget next year. "I'm quite sure of it", says the ex-president of the space research agency CNES, Hubert Curien, who is Fabius's man at the ministry of research and technology. But in an interview, Curien had a message for all French scientists who — he was also sure — would "understand" that in difficult times for Europe and the world, they can expect nothing exceptional.

But M. Curien was one of the architects of the "law for research", voted two years ago, which promised an average real 17.8 per cent increase per year in the civil science budget and 4.5 per cent annual increases in research posts. Those figures were exceptional. Unfortunately, says Curien, today "the economic hypotheses on which those figures were based seem not to apply". But he promises to "do his best" for science, and has set his own targets. He sees it as "very urgent" to increase the computing power of French laboratories. French scientific computing has suffered because of an unresolved conflict between industrial policy (support of home industry) and scientific needs (foreign computers, as French industry has neglected the scientific market).

Curien's efforts may be hampered by the removal of industry from his ministerial responsibilities. The first research minister in President Francois Mitterrand's term, left-winger Jean-Pierre Chevenement, built up the ministry from a small office of the Prime Minister to a "super ministry" of research and industry. When Fabius took over from Chevenement (who resigned over economic policy), he inverted the title to "industry and research" and now, as Prime Minister, he has hived off industry and given research alone to Curien — who is more scientist, or science administrator, than politician. "Fabius wanted a technical man" to look after research, Curien explains, and felt that the industry portfolio, heavy with the problems of the coal and steel industries, needed a separate ministry. However, this might be thought to mark a political downgrading of science, and a potential conflict between industrial and research interest (as in computing). Against this must be set the fact that Fabius has confirmed his belief in research and technology as the potential saviours of France. The 1982 law for science aimed to make French research and development spending reach 2.5 per cent by 1985 (it bottomed out at 1.8 per cent in the late 1970s.) It will not do so, Fabius has admitted, but he has raised the stakes by announcing a longer-term (wisely undated) target of 3 per cent.

Curien's main objective is "quite clear".

Despite the split of his ministry from industry, he seeks to improve the links between industry and research. "The volume of industrial research is not enough", he says. Curien seeks new means to stimulate it — through the banks (now nationalized) or by fiscal incentives.

Curien also plans to bring to a rapid conclusion the lengthy Gallic negotiations with trades unions and others over the "statute" or regulations defining careers in research, and is looking for help from Fabius on the matter; and he wants to stimulate the mobility of scientists, not so much geographically as among disciplines.

Agent Orange

Australian study continues

Canberra

FOLLOWING a cabinet decision not to support the full morbidity study requested by the royal commissioner, Mr Justice Evatt, the Australian Government announced on 7 August its intention to extend the Royal Commission into the effects of Agent Orange on Australian veterans of the Vietnam war. Citing an expected cost increase from \$A6 million to \$A10 million as a major reason for the abandonment of the morbidity study, the government has extended the time for the commission to report to 30 April 1985.

This is the latest in a series of investigations during the past five years arising initially from anecdotal claims by Australian Vietnam veterans that their health had been severely undermined by wartime exposure to various pesticides, in particular, the defoliant Agent Orange, a 50:50 mixture of the herbicides 2,4-D and 2,4,5-T, including the contaminant TCDD (2,3,7,8-tetrachlorodibenzo-*para*-dioxin). The veterans claim that during the 50,000-man Australian involvement in Vietnam, from 1962 to 1972, they were sprayed by their own forces and by the aircraft of Operation Ranch Hand, the US defoliant programme.

Newspaper reports of claims began to appear in 1979 with accounts of cancer, depression and anxiety states, and sudden uncontrollable rages among other complaints. In early 1980, weary of being branded malingerers and drug addicts by government doctors, unsuccessful claimants on the repatriation system organized themselves in the (Australian) Vietnam Veterans Association (VVA) and now represent approximately 10,000 of the troops who served in Vietnam. In 1982, a Senate standing committee into pesticides and the health of Vietnam veterans found that, while Australian troops were likely to have been exposed to insecticides, army records demonstrated that herbicide exposure could not have taken place: the

The new minister also gives research training a high priority. A new system for awarding doctorates, shorter and more like PhDs than before, gets his full approval. But he would like to arrange for many more doctoral students to do their research in industry, while retaining their university links, and he wishes to have a better match between the subjects of doctoral theses and the needs of industry, not so much for the results as for the training this will provide. This will be a proposal that may well find a response at the ministry of education, where the new minister is law-for-science man Jean-Pierre Chevenement. But he may be so swamped by the vexed problems of private and public schooling in France that he will have little time to spend on university affairs.

Robert Walgate

veterans' symptoms were more likely to be due to delayed combat stress, the committee claimed.

The Royal Commission began work in March 1983, but the cabinet was obliged to reconsider the feasibility of future investigations when in March this year VVA withdrew support for a pilot study of veterans' health, stressing its desire for a speedy resolution to the veterans' claims through the repatriation system, where the burden of proof for service-related injury is usually small. Meanwhile, several thousand Australian veterans and their families await the outcome of the \$180 million US settlement, although this suit refers only to Agent Orange and VVA is not directly involved.

Jeffrey Sellar

History preserved



THE zoological drawings from Captain James Cook's three voyages to the Pacific between 1768 and 1780 are being restored and conserved by the British Museum (Natural History) with the support of IBM (United Kingdom) Limited. □

Acid rain

Allies for UK Government

THREE British reports on acid rain in the past two weeks, in what newspapers call the "silly season", might seem a neat way to bury an issue. But that might not have been the intention of the Watt Committee on Energy, the House of Lords Select Committee on the European Communities or the Royal Society (see below). All emphasize the scientific complexity of the problem and none offers a quick palliative.

And yet, the Department of Energy is so delighted with the independent Watt Committee report that it has offered to pay its acid rain working party, under the chairmanship of environmental scientist Professor Kenneth Mellanby, to spend another six months expanding its review of policy options.

The reasons for government glee may not be far to seek. Britain is a large net exporter of sulphur dioxide, the long-range component of acid rain, and is under pressure from European partners to retrofit expensive sulphur scrubbers to its 20

major coal-fired power stations. But the Watt Committee working party has armed the government with some strong arguments to resist that pressure. Similarly, the House of Lords committee offers reasoned resistance to the proposed EEC directives.

The Watt Committee argues, for example, that the liming of exposed lakes may be the most effective means of control for acidified lakes, and may cost only "a few per cent" of measures to retrofit power stations.

The Watt Committee also points out that NO_x and hydrocarbon emissions, which in photochemical reactions produce ozone and oxidize sulphur dioxide to sulphur trioxide, greatly increasing sulphur solubility, may play a controlling role in producing acid rain. Thus whereas a decrease in NO_x and hydrocarbon emissions might have a proportional effect on acid rain, a decrease in sulphur dioxide emissions might not be so effective. Moreover, NO and HC are, on the whole, emitted and deposited locally.

And apart from all that, the committee also says that countries complaining of net import of sulphur dioxide could more effectively reduce acid precipitation by reducing their own outputs which, being local, have more effect. Thus, says the report, to reduce sulphur deposition in Norway by one ton, Britain would have to reduce its output by 46 tons; but Norway would have to reduce its emissions by only two tons. Thus it would seem advantageous for Britain to pay complainants to clean up their own doorsteps, while continuing to emit sulphur dioxide unhindered.

However, the Watt Committee working party, in other sections of its report, emphasizes that there is so much uncertainty that it is simply unclear what strategy would be best to solve an admitted environmental problem. "In Britain, when we know what is the cause of a problem, we do have a good record of cleaning it up", said Mellanby. He was just afraid that millions of pounds will be spent on sulphur dioxide control to little effect, diverting funds from more effective projects.

Meanwhile the House of Lords slammed into the proposed EEC legislation, which would aim to reduce large power station emissions of sulphur dioxide by 60 per cent, particulates by 40 per cent and nitrogen dioxide by 40 per cent, by 1995. Just to achieve the target for sulphur dioxide would cost the British electricity industry £1,990 million in capital and £350 million in running costs, the committee estimates. But the European Commission has underestimated these costs by a factor of five, and "substantially overestimated" the value of the benefits "mainly due to a misunderstanding of the causes of alleged damage by pollutants". Desulphurization

would also cause environmental damage: four million tons of calcium carbonate would have to be excavated each year and 7.5 million tons of wet calcium sulphate disposed of, only some of it reliably.

The report also criticizes the Commission for misinterpreting the recent US National Academy of Sciences report on acid rain — which in part reviewed European data (from the European Monitoring and Evaluation Project, EMAP, which involves over 106 stations). This data, as the NAS report demonstrated, showed no evidence of a linear relation between sulphur dioxide and NO_x emissions. Yet the commission focused on the single US source of data, the Hubbard Brook site, which evidence has been strongly criticized. The House of Lords says bluntly: "the Committee are disturbed that the Commission has weakened confidence in its directive by misrepresenting the evidence on such a critically important issue".

Nevertheless, the House of Lords argues that some control measures should be taken — it recommends the long-term introduction of pressurized fluidized-bed combustors, which are more energy efficient and less polluting, and also suggest that "immediate steps" be taken to fit two power stations with flue gas desulphurization. (The EEC regulation would imply the same be done to 12 stations.) The result would be a 30 per cent reduction in sulphur dioxide by the year 2000, according to the Central Electricity Generating Board.

Robert Walgate

The three reports are: *Acid Rain*, The Watt Committee on Energy, Report No. 14; *Air Pollution*, 22nd report of the House of Lords Select Committee on the European Communities; *The Current Status of Research on Acidification of Surface Waters*, by B J Mason, The Royal Society.

Mason's own view

SIR John Mason, according to some observers, has committed a slight *faux pas* in releasing his personal review of the status of "acidification of surface water research" (published by the Royal Society). Sir John is programme director of the tripartite Royal Society / Norwegian Academy/Swedish Academy research project, and he may have embarrassed his partners who wish to remain objective — and uninfluenced by British views.

However, Sir John makes it clear that his report is personal, just one paper submitted for discussion at an early management meeting of the tripartite study, and not a preliminary report of the study itself. Politically, his most important (preliminary) conclusion may be that the rate of production of sulphuric acid, and probably nitric acid, in rain "is not proportional to the emission or atmospheric concentration of the precursor gases sulphur dioxide and NO_x , but is limited by the availability of oxidants such as HO , H_2O_2 and O_3 ". This is the question that the House of Lords committee studying acid rain (see above) consider could be a decisive factor in a political decision to reduce emissions.

As for the tripartite study itself, Sir John said last week that he is touring research groups "flying round in a small plane" identifying gaps in research and attempting to match facilities and people. He expected the £5 million research programme to be announced in December, and there is agreement that a few big, site-specific, international studies should be made. "I don't want penny packets", he said.

Robert Walgate

Student benefits

THE Department of Education and Science has announced a series of improvements in the financial support British students may expect in the coming year. These include an increase in the employer contribution a student may receive before the state-backed maintenance grant is cut. Students may now receive a total of £1,600 from all sources — £1,900 for National Engineering Scholarship Students. (The current student lodging grant is £2,100 a year for London and £1,435 for elsewhere.) The government hopes that these concessions will encourage students to pursue careers in industries vital to the economy.

Other students to benefit are those studying outside Britain and those undergoing teacher training while employed as full-time teachers. There are also to be increases for students' dependants.

The National Union of Students, welcoming any change that will leave its members better off, says that it will continue to push for an adequate student grant across the board. **Marcus Chown**

Mt Wilson 100-inch telescope

SIR — Your article¹ referring to the closure of the 100-inch telescope at Mt Wilson Observatory cites some disparaging opinions of the value and usefulness of the telescope. We would like to present another point of view.

The 100-inch reflector, despite its age (it has been in service since 1917 and was for thirty years the largest telescope in the world), is one of the finest instruments in existence. The high optical quality of its mirrors enables it to take full advantage of the excellent seeing often enjoyed at Mt Wilson — an aspect of the site not remarked upon in the news note. Star images seen in the Mt Wilson telescope are not infrequently smaller and crisper than any we have seen in other telescopes. Substantial and successful efforts have been made in recent years to obtain first-class reflective coatings on the telescope's mirrors — not an easy thing to achieve on large surfaces. The sharpness of the images and the high transmission factor of the telescope combine to make the 100-inch an extremely potent instrument. Its advantages are especially apparent at the Coudé focus (the stationary focus at which particularly heavy or delicate equipment needs to be mounted). Not only is the reflective power of the mirrors good, but intelligent design has required only three successive mirrors in the optical train; more "modern" designs call for five (as in the Anglo-Australian telescope) or even seven (as in the Isaac Newton telescope), resulting in disastrous loss of efficiency.

Its auxiliary equipment is also of the highest class. The stellar spectrograph at the Coudé focus is superb. The data from some of the photographic spectrograms we have obtained with it have been presented as photometric "atlases" of the spectra of two representative stars^{2,3}; in terms of spectral resolution, signal/noise and wavelength coverage these are the only works of their kind for any star apart from the Sun, despite the demand for many more such atlases.

There is no equipment on any telescope accessible through the Science and Engineering Research Council (SERC) that will enable spectra to be recorded with anything like the quality and detail available from the 100-inch Coudé spectrograph. In the past four years alone, we have made nine visits to California at SERC's expense to obtain spectroscopic material at the 100-inch. Coudé spectrographs comparable with that at Mt Wilson were planned for both the Anglo-Australian and Isaac Newton telescopes, but the former was cancelled when the design was complete and the latter is in a state of abeyance despite being half-built. It is most regrettable that there is not, and never has been, any provision for high-quality spectroscopy on SERC telescopes: a generation of astronomers has grown up in

this country without any opportunity to learn or to appreciate a large and important area of astronomical research. Whether observational work on extra-galactic objects is "more exciting", as your correspondent asserts, is a very subjective matter.

Your remarks about light pollution of the Mt Wilson site are only partly justified. We should point out that the notorious Los Angeles smog never affects night-time observing, since at night there is invariably a temperature inversion which places an effective lid on the smog bowl well below the level of the mountain. The sky, certainly, is not as dark as it is at a site remote from cities; but even at the best sites half of the observing time is similarly compromised by moonlight. Full moonlight is much brighter than the Los Angeles light pollution at Mt Wilson. Yet it has never been suggested that good telescopes should be shut down around full Moon; indeed, even "bright time" is in heavy demand, particularly since instrumental developments (some of them made at Mt Wilson) provided equipment which permits subtraction of the sky contribution from spectra contaminated by a bright sky.

In short, we wish to suggest that the 100-inch telescope is being closed not because of any shortcomings of its own, its site or its staff but because of financial stringency within the Carnegie Institution. It will be as amazing as it will be incomprehensible if organizations suffering from the acute shortage of observing time on good large telescopes do not see in the present predicament an opportunity to buy the use of a very effective facility for a very modest operating cost.

R. & R. GRIFFIN

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Cambridge, UK*

1. Budiansky, S. *Nature* 310, 88 (1984).
2. Griffin, R. F. *A Photometric Atlas of the Spectrum of Arcturus* (Cambridge Philosophical Society, Cambridge, 1968).
3. Griffin, R. & R. A. *A Photometric Atlas of the Spectrum of Procyon* (published by the authors, Cambridge, 1979).

Kitt Peak

SIR — In your news item on the reorganization of Kitt Peak (*Nature* 12 July, p.88), you state incorrectly that Dr Geoffrey Burbidge and Dr Jack Zirker had been reappointed as directors of AURA's Kitt Peak National Observatory and the National Solar Observatory, respectively, for five-year terms in 1982. In fact, both contracts had been renewed only until such time as the proposed National Optical Astronomy Observatories (NOAO) reorganization took effect, without prejudice to either director's possible candidacy for the directorships of those divisions of NOAO. Incidentally, Dr Zirker did not apply for the

position as Associate Director of NOAO for the National Solar Observatory.

I find it disappointing that your article gives the impression that AURA invalidated its contracts with Burbidge and Zirker as observatory directors. Both understood the terms of their contract renewals. I feel it is particularly unfortunate that our international colleagues may have gotten the impression that there are clouds over the careers of these two fine astronomers, each of whom has made major contributions to astronomy as an observatory director, and who will no doubt continue to do so in pursuit of their individual research.

JOHN M. TEEM
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Basso continuo

SIR — The intriguing association between music and nucleic acid chemistry¹ may generate the need for new terminology. Perhaps nucleotide sequences should, from now on, be notated on the *base clef*.

MARTIN F. HEYWORTH

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1. Hayashi, K. & Munakata, N. *Nature* 310, 96 (1984).

Jumping the gun

SIR — Restrictive editorial policies concerning the announcement of a scientific discovery in the popular press before formal publication, such as those embraced by John Maddox (*Nature*, 21 June, p.665), only inhibit the timely exchange of information between scientists and the public. *Physical Review Letters* should be applauded, not condemned, for its adoption of a liberal and enlightened policy.

Maddox seems to be unaware that the growing corps of increasingly qualified scientific journalists will, despite embargos and edicts from journals, ferret out stories through personal contacts, preprints and other means. His case for restricting advance announcements, to prevent misrepresentation of discoveries to the public, does not hold up. Even after formal publication, results can be misinterpreted or sensationalized.

It seems to me that the public has a proprietary right to know the results of research whenever it is conducted with public funds. This issue, in particular, needs testing in the courts. Restrictive policies such as those adopted by *Nature* will hasten the day that test comes about.

LEIF J. ROBINSON

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The geology of nuclear waste disposal

W.F. Fyfe*, V. Babuska†, N.J. Price‡, E. Schmid§, C.F. Tsang||, S. Uyeda¶ & B. Velde#

An ICSU committee on the geological disposal of high-level radioactive wastes has concluded that century-long interim storage is essential and that disposal in subduction trenches and ocean sediments deserves more attention.

IN 1978, the International Council of Scientific Unions (ICSU) formed a series of working groups to consider the global problem of the disposal of high-level nuclear wastes. This committee, while involved with continental waste disposal, was naturally led to compare continental with oceanic sites. The global problem is impressive. About 10 per cent of the world's electrical energy supply is now nuclear and the percentage is growing. Developments in Europe¹ and Japan reinforce the general conclusions from the Club of Rome², "at present the only alternative energy source to fossil fuels which is both technically feasible and economically viable is nuclear fission".

For energy planners to reach the best possible compromises between energy production and environmental deterioration, clear demonstrations that nuclear wastes can be safely stored are necessary. But nuclear wastes may be dangerous for at least thousands of years, so all demonstrations must rely on indirect scientific arguments and predictions. This is why geologists must be involved for they, more than others, can demonstrate that sensitive materials are preserved in certain rocks and not in others.

If there have been problems and time delays in providing the necessary answers, it must be remembered that scientists have never before been asked to answer disposal problems on this time scale. Further, the problems are not unique to the disposal of nuclear wastes, for there are others (mercury, cadmium, lead, etc.) which have infinite lifetimes. Only in the recent decades has man become capable of modifying the surface environment of our planet to such an extent that such problems have reached critical importance³.

The basic questions that arose in our deliberations were these:

- Is secure storage possible for periods of thousands of years?
- What is the optimum period of interim surface storage?
- Where and in what rock types is the optimum site for disposal?
- Is present geotechnology adequate, or must new technology be developed for disposal engineering?

A major radwaste (radioactive waste) repository might involve a cavity of the order of 10^7 m^3 (0.01 km^3). Do we have evidence that comparable volumes of sensitive (soluble) natural materials have been preserved intact for periods of millions of years? The clear demonstration of integrity in natural systems must be one of the most important aspects of discussions on site selection⁴.

Natural analogies

Perhaps the ideal demonstration might come from a natural fission reactor such as that at the Oklo site^{5,6}. This natural fission reactor, which operated about two billion years ago, does indeed demonstrate an amazing local retention of uranium, species derived from plutonium and species such as technetium, but others, such as iodine and caesium, are largely missing or dispersed. Oklo is a little too old, and perhaps can be used to demonstrate only a worst case.

The large-scale preservation of bedded salt deposits dominated by highly soluble minerals in rocks of a wide variety of ages, including massive units of Precambrian age in the Salt Range of Pakistan, may be more relevant. The preservation of relatively thin (100 m) salt beds for periods exceeding 10^8 years provides time-integrated water/rock ratios which cannot have exceeded 2:1, for the solubility of NaCl at 100°C is about 40 per cent by weight. Data on salt dome solution rates⁷ at depths of ~ 500 m show very small solution rates with pore-water velocities of $3 \times 10^{-7} \text{ km per year}$.

Perhaps the best long-term permeability

data for moderately deep systems are to be derived from older rocks carrying significant deposits of oil and gas. Such rocks are invariably of sedimentary origin, and it is for sediments that the most reliable data on fluid flow are at present to be found.

Natural examples of sensitive materials abound. Many oxide and sulphide mineral ore deposits can react with oxidizing or reducing fluids. During any such reaction, trace metals will be redistributed. The preservation and lack of corrosion in native copper deposits over a billion years old (as in the famous deposits of Michigan) is also impressive. Useful examples abound, but sufficiently detailed studies of such phenomena are rare.

Interim storage

High-level radioactive wastes produce heat. Heat production from short-lived isotopes falls an order of magnitude per decade for most spent fuels^{8,9}. Thus, a CANDU fuel bundle produces 2,000 W after it leaves the reactor, 60 W after one year and 1 W after 100 years⁸. Swedish spent fuel produces (per tonne of U) 10^4 W after 1 year, 10^3 W after 10 years and 10 W after 1,000 years⁹.

Because of plate tectonic and erosional phenomena, most large continental blocks are subjected to stress at levels which vary with location and depth. When near-surface rocks are sampled, relatively new cracks are generally found. In preparing a given site for disposal of waste, local stresses will be generated by mining, but after emplacement of waste, thermal stresses may build up for a long period of time. The perturbed thermal gradients around the waste repository may produce new mechanical cracking and even hydrofracturing by expansion of pore fluids. Such problems are not yet well quantified.

It is certain that these effects would be reduced if interim storage were lengthened, while any influence of radiation on containers and back-fill as well as danger to workers would all be reduced. Studies on radiation damage to wastes are still inadequate¹⁰. The ICSU group

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considered that interim storage should be for the longest acceptable period, because technology for final disposal, whether terrestrial or on the seabed, is not yet established. The "take time" or "waiting" option¹¹ might be more responsible than a best-guess quick alternative. And, in any case, in a hundred years, wastes may be put to uses we do not yet contemplate. We conclude that there is a need for a major effort to find sound methods for secure long-term (>100 years) interim storage of radwaste which are directly observable and which can be monitored. Such storage would virtually eliminate the problem of thermal stresses and would allow closer packing in a repository and the more sophisticated engineering of smaller cavities.

Crustal stability

Hitherto, the most intensive investigations of site location have centred on continental crust. Much less effort has been devoted to seabed disposal, but we would agree with Hollister *et al.*¹² in their conclusion that disposal of submarine clay formations also requires rigorous assessment. The structure of seafloor crust is less complicated, while its dynamics and thermal regime are better known than for continental crust. The permeability of seafloor crust is more predictable^{12,13}.

New observations on the structure and processes near ocean trenches suggest that subduction zones require careful consideration as potential disposal sites. Data from trench environments (see refs 14,15) clearly show that in most, the lithosphere cracks to form a horst and graben structure (Fig. 1) and that light sediments are carried down with the descending plate. Most trench environments are extremely cold and are characterized by the lowest thermal gradients on the planet, so that solution processes of waste or corrosion of containers will be minimal. Moreover, the total mass of fluid available for transport can be quantified, as the material is moving into essentially anhydrous upper mantle¹⁶. Another advantage of a subduction graben is that the structures are so large that there are virtually no restrictions on the size or shape of an object for disposal. Hence, the trench option requires careful evaluation.

On the continental crust, perhaps the first requirement for the site of a major repository is that there should be volumes of the order of 10 km³ with minimal predictable permeability and with a record that suggests no potential disturbance or permeability change for the next 10⁶ years.

On a global scale, present understanding of plate tectonics clearly shows that the Earth's crust can be divided into zones near plate boundaries and the much larger intra-plate regions. Most plate boundaries and rift zones are associated with major volcanic and seismic events. While plate boundary regions clearly change over geological time (on scales >10⁸ years), for

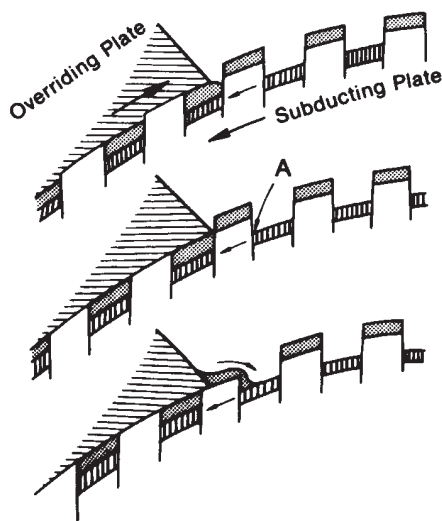


Fig. 1 Horst and graben structures frequently observed in trench environments¹⁵. Nuclear waste placed on a site such as A in soft sediments would later be covered by scraped-off sediments and subducted at a rate of 2–10 cm per year.

the purpose of waste disposal, the present global configuration can be considered a steady state.

Our record of seismic events, however, is quite short and cannot always be used to define stable regions. The Korean Peninsula is at present aseismic, but historical records show high activity a few centuries ago¹⁷. In any case, while seismic activity is a good indicator of stresses of a certain magnitude, it says nothing of stress below the seismogenic level that may, nevertheless, be significant. It is essential that accurate *in situ* stress measurements are made early in site evaluation.

Studies of Quaternary deposits in a given region provide the most convincing evidence for stability over the past 10⁶ years or so. If a region with Quaternary cover shows no recent displacements, confidence in stability must be increased. In principle, cracks, faults and the like can be dated by careful study of the new minerals formed in them, by using stable isotopes (for example, ¹⁸O/¹⁶O) and by other geochemical observations (for example, sulphide oxidation) it may even be possible to estimate the minimum total fluid flux through the cracks and faults. But few data of this type are available¹⁸.

The common geological wisdom is that stable regions with old rocks should be better for radwaste disposal than young orogenic regions, but comparisons of granitic regions in Japan and Sweden show that this is not necessarily true¹⁹. There is a tendency for rocks in ancient terranes to show the most complex fracture history; the Cretaceous granites of Japan have domains similar to Precambrian granites of Sweden.

The most important property of a rock mass in the radwaste disposal context is low permeability. Permeability in granitic

rocks is that related to cracks and faults, the former being more important to near-field considerations, the latter being more important in far-field considerations. In a rock mass there are concentrated zones of cracks and faults in which are often found the large-scale faults. Between these concentrated zones, even in Japan, there is often a block almost free from cracks and faults, and it may be possible to locate a mass with a dimension suitable for radwaste repository, say 1–3 km in diameter. If we compare such a good block of granite in Japan with a common block of granite in Sweden, the former is not necessarily inferior for disposal purposes.

In Japan, the primary stress agent is tectonic, due to plate convergence, whereas in Sweden, it appears to be related to epeirogenic movements due to glacial unloading. Cracks in Japanese granites tend to be steeply dipping conjugate pairs, whereas Swedish granites also have an abundance of horizontal sheet cracks to depths of several hundred metres²⁰. In Swedish granites, the stress measurements indicate that the horizontal stress (remnant of glacial loading?) is 1.5 times the vertical stress and the maximum rate of uplift amounts to almost 1 cm per year. Although Japan is in an orogenic zone, a few *in situ* measurements in tunnels and underground power plant sites indicate that the ratio of horizontal to vertical stresses is in the range 0.7–1.4 and that the maximum rate of vertical movement is only about 1 mm per year. Moreover, the filling of cracks by clayey materials appears to make the Japanese granite much less permeable and more adsorptive than the Swedish granite at similar depths. Although this comparison is admittedly quite cursory, it provides some good reasons why further investigations should be worthwhile even in a geological region such as Japan.

Seismic techniques seem to provide promising tools for the qualification of crystalline rock bodies against the presence of cracks and fracture zones²¹. So-called cross-hole methods allow sampling of rock to a distance of about 1 km in various directions, and can be extended to three-dimensional surveys, enabling the velocity anisotropy to be determined and the crack parameters to be estimated spatially^{22–24}. The electrical conductivity of dry rocks is also a function of the crack density parameter, but in saturated systems additional parameters have to be considered.

Rock fractures

Most rocks at depth tend to become more impermeable and, indeed, fractures should theoretically close or seal at depth, but this is not always observed²⁵. Price²⁶ has shown how rebound from ice retreat can cause fracturing, and recent Canadian and Swedish studies^{20,21,27,28} show the great complexity of fracture systems in crystalline rocks. On the positive side, Mair and Green's work²¹ shows that modern seismic

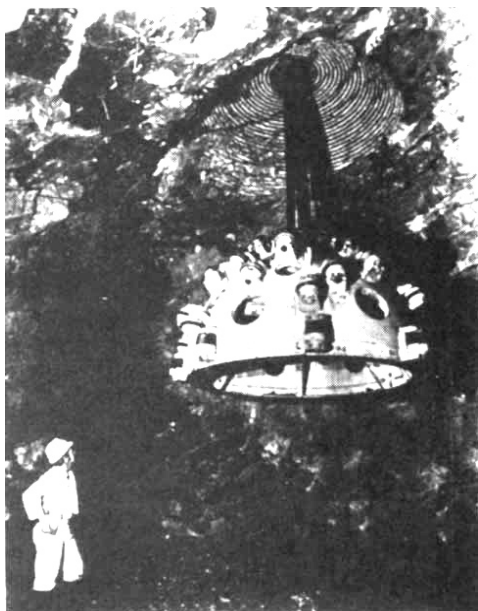
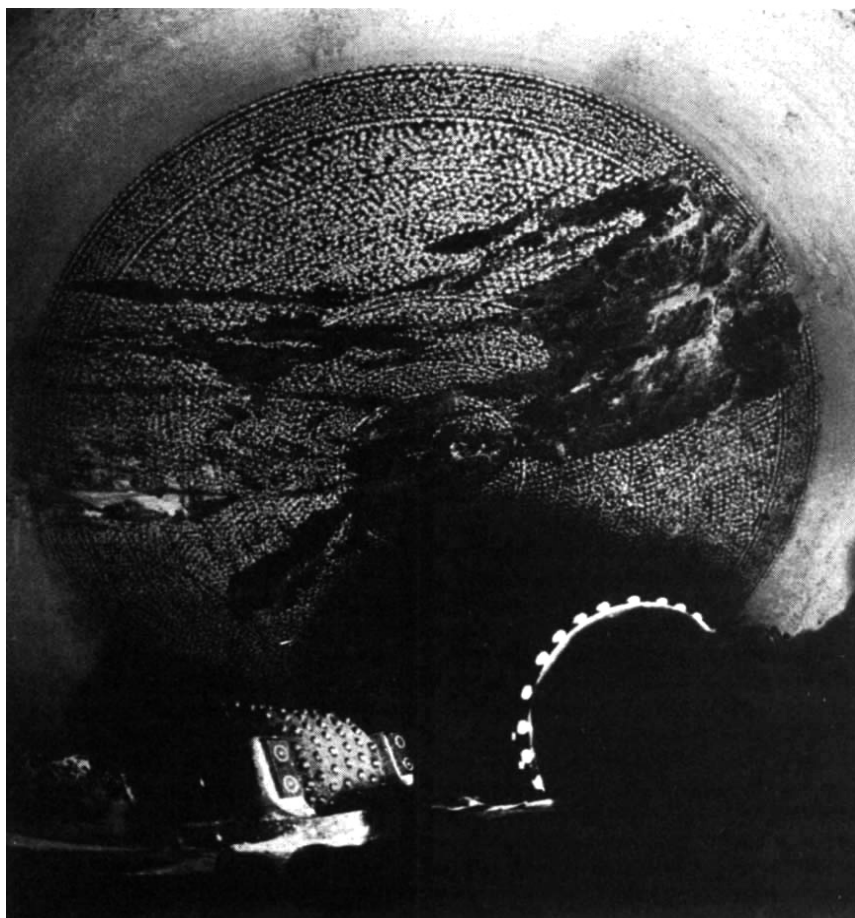


Fig. 2 Modern boring machines at work in hard rocks (quartz conglomerates) in the gold mines of Johannesburg. Such technology produces minimum damage to the rock mass (compared with drilling and blasting) and produces smoothly regular walls into which prefabricated waste packages could be introduced with minimal complexity and handling.



techniques can be used to detect major fractures.

In general, the relative ease of migration of groundwater is determined by both the intrinsic permeability of the unfractured rock and the fractures that exist within or which cut the rock mass. The flow rate in fractures is usually markedly higher than through the porous media of the unfractured rock. With few exceptions, rocks of all types and which have experienced a variety of geological environments are cut by fractures. The variety of existing rock types, and the various degrees of induration, compaction metamorphism, cooling, deformation, etc., which they have experienced, precludes any simple assessment of the multitude of possible fracture systems which occur.

It is instructive to consider a simple hypothetical example. Assume that three rock types (granite, sandstone and shale) have experienced the same degree of extension (say 10^{-3} , or 0.1 per cent) and that they have all reacted to the extension by developing sets of barren extension fractures (joints). These fractures may, on average, be spaced 10 m apart in the granite. If all the extensile strain is relieved by opening of the extension fractures, then each fracture will have an average width of 1 cm. A moderately thick sandstone bed, on the other hand, may be cut by fractures which have an average separation of 1 m, so that individual fractures would have a width of about 1 mm. And a relatively thin

shale may have a fracture spacing of only 10 cm, with a fracture width of 0.1 mm.

Thus, in a given traverse perpendicular to the trend of the fractures, the shale will have a hundred times as many fractures as the granite. But if all other flow parameters are constant, the rate of fluid flow through a fracture is related to the cube of the width of the fracture. Hence, the flow rate through a single fracture in the granite is potentially 10^6 times faster than through a fracture in the shale, even though there are 10^2 more fractures in the shale. The flow rate in the granite is potentially 10^4 times faster than through the shale.

One therefore arrives at the conclusion that rocks cut by a myriad of small fractures may be better aquifers than those which are cut by relatively few fractures. They will certainly be superior for all dispersion, dilution and absorption processes. But fracture systematics are not well studied and are even less well understood²⁹. Of all waste disposal problems, understanding the hydrological characteristics of fracture systems is one of the most urgent areas for study.

Although much attention has been paid to thermal perturbations on the transport parameters of species from repository to the surface, the present state of modelling is imperfect³⁰. In a repository, all major transport processes (heat flow, chemical flow, water flow) are coupled to the mechanical properties of the rock (stress corrosion, hydration expansion and the

like). While some of these couplings are well modelled, fully coupled models are inadequate³⁰⁻³⁶. In particular, fractured media are not adequately treated and validation of all models against field data is essential.

There is no doubt that there is no single ideal host rock for radwaste disposal^{18,37}. Massive isotropic rock masses may tend to develop fractures of wide aperture; bedded sediments may be best for retention and dispersion; rocks formed at high temperatures may tend to suffer the greatest change in properties by the motion of low-temperature fluids. A compromise might be to use an igneous rock mass covered by a well-layered flat-lying sedimentary sequence to guarantee dispersion should leakage occur from a pluton (see also ref.38). At present we know more about flow in sediments than in any other rock type.

As to waste forms, containers and back-fill materials, it must be kept in mind that all are related and must be as compatible as possible. We commend the Swedish effort on containers, for if the container life can be guaranteed for 10^3 – 10^6 yr, hazards are greatly reduced. Attention should focus on materials such as copper³⁹ and corundum⁴⁰, for which there is excellent control of natural solution and corrosion in geological situations.

Proposed waste forms vary from fuel rods themselves to glass and crystalline materials^{25,37,41}. There is a need for careful

work on the reactions of these materials with wet back-fill materials. Most proposed materials are made at elevated temperatures and are thus likely to be unstable in low-temperature regimes.

Almost all systems propose that swelling smectite clays will be used as back-fill, with an array of possible additives to control oxidation-reduction conditions and to retard specific problem elements. There is a great need for further study of the geological stability of such materials and of the influence of radiation on waste forms¹⁰. Little is known about the kinetics of bentonite → illite or illite → kaolinite processes, but the best data may be geological.

Mining technology

Although much effort has been expended on many aspects of radwaste disposal, little attention has focused on mining technology. Again, long-term temporary storage should greatly reduce the necessary repository volumes. Our expert colleagues note that:

- Boring machines may provide the best cavities with least damage (Fig. 2).
- Appropriate mining technology for depths up to 4 km exists, and the 500–1,000-m depths commonly considered adequate require careful justification.
- Repository design should follow site and rock type selection and a full analysis of all rock properties. Until such data are available, designing will tend to be inadequate.
- All excavations must be kept as small as possible. The zone of a rock mass influenced by an excavation increases in size with at least the square, and possibly the cube, of the largest dimension of the excavation.
- Containers, handling technology, etc., should be designed for the excavation and not vice versa. (Most present approaches are quite the opposite.) Problems of ground control are exponentially related to excavation size.
- Satellite layout may be the most suitable. Individual areas could be small, easily isolated and monitored. Each satellite could be sited to use the best local conditions and the best technology.
- The development of techniques to see discontinuities (faults, dykes) ahead of mining operations should be accelerated. Present crack-sealing techniques require careful reconsideration; some clay grouts may promote later slip.
- Optimum depth may become apparent only after a full-scale study of rock properties, stress levels, etc., in a given region.
- Experience shows that assessing closure (collapse) is difficult and inconclusive. It is important to separate elastic closure from that due to dilation of fractured rock. The interactions between fractured and intact rock are poorly understood.
- Stress levels are more predictable and more regular at great depths. Near the surface, unusual or unexpected results are not uncommon.

Future integrity

The future integrity of a repository may be directly affected by man through drilling during exploration for minerals or for hydrocarbons or in geothermal energy projects. There may also be indirect effects because of major engineering construction such as the building of a major reservoir, as seismic activity is often associated with such constructions.

Ideally, a repository should be sited in an area completely free from seismic activity, but the identification of such areas is probably impossible.

Climatic changes and their effects should be taken into account. Perhaps the most probable major climatic event which can occur within the next 10^4 – 10^6 yr is the onset of a major glaciation, with significant advances of polar and local ice sheets. The advance and retreat of such ice sheets would have a variety of effects, including seismicity, fracturing and changes in the hydrogeological regime^{20,21}.

Finally, we note that a repository could be breached by a meteoric impact. This subject tends to be ignored, but the chances of such an event may not be as small as some believe^{42,43}. What we emphasize here is that there is safety with depth, as shown by analysis of impact phenomena^{40,44}. And most of these perturbations do not apply to deep ocean situations.

Conclusions

The chief conclusions of our study are as follows.

- We consider that interim storage of radwaste for periods of 100 years is desirable.

● Most work on radwaste disposal so far has been laboratory work. We consider that too little effort is being put into geological studies related to proposed methods, materials, etc.

● The best indications of tectonic stability, long-term permeability, influence of climatic changes, etc., can be found by studying deposits formed over the past 5 million years or so that cover potential host rocks.

● Present data on fracture systematics of rocks and their relation to permeabilities on all scales are quite inadequate.

● There is a great need for special studies of optimum mining technologies.

● Studies of the influence of thermal perturbations and the coupling to fluid flow, rock alteration and fluid chemistry are only now developing.

● New data on the subduction process appear to make this alternative worthy of careful analysis; the same is true of ocean floor disposal.

● There is a great need for accelerated development of underground laboratories in all proposed rock types.

● There is a need to consider the problem of how a major leak from a repository might be detected and treated.

We acknowledge helpful discussions with representatives of the US Department of Energy, the UK Department of the Environment and Atomic Energy of Canada Ltd., who made themselves available to answer specific questions. We particularly thank the Ontario Hydro personnel who allowed us access to nuclear power plants and interim storage facilities.

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Malaria vaccine in sight?

The latest development in the molecular biology of malaria parasites is not so much a promise of prophylaxis as a sign that the promise will not forever be postponed.

WHAT a day to meet! Thus Dr Adetokumbo Lucas, director of the World Health Organization's Special Programme for Research and Training in Tropical Diseases, brandishing one day last week photocopies of a set of page proofs of the articles in the current issue of *Science* (10 August) describing the structure of the gene for the principal antigen of the sporozoite form of human malaria, caused by the parasite *Plasmodium falciparum*. What Lucas meant was that since Nussenzweig and his associates at New York University last year described the structure of the corresponding antigen of monkey malaria (*Nature* 305, 29; 1983), everybody has been waiting for the other shoe to fall. And now it has.

As always, even the expected is surprising. Of the work now published, the more complete is that reported from the National Institute of Allergy and Infectious Diseases, the National Cancer Institute and the Walter Reed Army Institute of Research in the United States (J.B. Dame *et al.*, *Science* 225, 593; 1984), but the New York University (NYU) group (V. Enea *et al.*, *Science* 225, 628; 1984) concurs in the chief finding — that the most conspicuous feature of the circumsporozoite (CS) protein from *P. falciparum* is a long stretch in which a sequence of four amino acids is repeated end to end. Repetition on this pattern marks out the structure of the two CS molecules whose structure has previously been inferred (from nucleotide sequences) — those of *P. knowlesi* and *P. cynomolgi*. But there the repeating units are respectively 12 and 11 amino acids long. That the repeating units in the antigenic coat of *P. falciparum* should be so short is one surprise. There will now be a great rush to tell what can be said of the presumed repetitive amino acid sequences in other species of *Plasmodium*, causing malaria in people and animals.

As now described by Dame *et al.*, the CS gene of *P. falciparum* is in three sections — a beginning, a middle and an end. The characteristic repeating section is the middle, which consists of no less than 41 four-codon repeats, corresponding to the amino acid sequence asparagine-alanine-asparagine-proline. But four of the 41 repeating units have a different structure, corresponding to the tetrapeptides asparagine-valine-aspartic acid-proline. In outline, the structure of the CS gene of *P. knowlesi* is similar but shorter, although the middle repeating unit

is shorter, at 12 twelve-codon units.

The beginning and the end of both genes differ from the central repeating stretch in carrying codons that would specify hydrophobic amino acids. The downstream end is assumed to be anchored in the outer cell membrane of the malaria parasite. Perhaps in real life, individual malaria parasites are protected from the outside world by sheaths of protein molecules whose stability depends not only on the way in which the molecules are anchored in the membrane but also on their sideways interaction with each other. It remains to be seen whether the model-builders will justify their existence by offering an explanation of what the three different repetitive nucleotide units now defined for malarial parasites imply about the presumed common function of the corresponding amino acid sequences. It must also be significant, but of something as yet unknown, that the repeating gene sequence is preceded and followed, both in *P. falciparum* and *P. knowlesi*, by closely similar regions (15 and 12 amino acids long respectively) whose similarity suggests a structural function to be identified.

Evolutionists will also prick up their ears at these developments. The conventional wisdom is that parasites such as *Plasmodium* are so exquisitely adapted to their hosts — replicating at their expense without utterly destroying them — that their evolution must have gone hand in hand with at least that of the primates. One minor issue is partly answered by Dame *et al.*: a gene with 42 four-codon repeats should be prone to instability in the sense that genetic recombination, insensitive as it is to anything but the chemistry of codons, will often decrease or increase the number of repetitive elements in a nucleotide sequence. But apart from the four odd units in the gene of *P. falciparum* described by Dame *et al.*, there are also 19 regular repeating units in which different nucleotide sequences code for the same amino acid sequence. Recombination has its hands tied behind its back. The NYU group says that the strain of *P. falciparum* they have investigated has a stretch of 23 identical repeating units, but there is no room for such a structure in the gene that Dame *et al.* describe. So is it possible that the details of the fourfold repetition are strain-specific? This appears to be the case with the blood stage antigen (Coppel *et al.*, *Nature* 306, 751; 1984).

Much will hang on the answer to that

question (which should be easily obtained). The potential importance of the discoveries now described depends to a large extent on observations that quite small portions of the CS proteins, hardly more than a dozen amino acids, are antigenic. So the great hope evoked by this latest development is that it may be possible to make vaccines against malaria by synthesizing an appropriate polypeptide. The snag is that if inter-strain variability is too flamboyant, the cocktail needed may be impossibly complicated. The immunogenic capacities of the nearly identical sequences before and after the repetitive sequences therefore cry out for study.

So how long will it be before there is a vaccine against malaria? The Lucases of this world know a trick question when they are asked one. The only proper answer, anyway, is "sometime in some future decade". Astonishing though it may seem, although malaria may be one of the best-described diseases, next to nothing is known, in modern language, of the mechanism of the details of the immune defence against it. Are antibodies, and the B-lymphocytes that secrete them, a sufficient defence against invasion by the sporozoites whose antigens are now described? How, artificially, will it be possible to give people a degree of protection against the infective form of the malaria parasite that will prevent lodgement in the liver? Is the cocktail-vaccine eventually needed bound to be one that caters not only for the antigenic variability of the invasive sporozoites but for the merozoites (matured in the liver) which eventually infect the red blood cells? And how, at the end of the cycle, can one prevent the maturation of the gametocytes from red blood cells, so breaking the cycle of infection via the mosquito?

Nobody knows. So we, but especially Dr Lucas, are in a cleft stick. In the endless battle against malaria, well-documented since Roman times and now said by the World Health Organization to be responsible for 150 million new cases a year (for want of an accurate number), we know that each new discovery may prove to be the basis for effective prophylaxis but with a chance of, say, one in 20. The work of Dame *et al.*, supported by the Nussenzweigs, is one of those 20. Time will tell whether discovery will be crucial. But as a pointer to the questions that need next to be answered, its value is already clear.

John Maddox

Scientific articles

A controllable aspect of the information explosion?

from Virginia Trimble

THE scientific information explosion needs no introduction. More journals are publishing more papers by more authors on ever-more-specialized topics than ever before. And, at least within the major American journals of astronomy and astrophysics, there are now also more words per paper than ever before (Abt, *H.A. Publs astr. Soc. Pac.* 93, 269; 1981). The rise in mean paper length started shortly after the Second World War, since when it has been monotonic and more than linear with time, with the result that a typical 1980 paper is nearly three times wordier than typical 1910 – 40 ones. A similar trend was recently identified in European and American astronomical letters journals (Harris, *W.E. Publs astr. Soc. Pac.* 95, 989; 1983). Despite editors' avowed intent to favour short communications over long ones, the average letter is 30 per cent longer today than it was 10 years ago. The obvious questions are why, and can anything be done about it? Determining just how widespread the phenomenon is and how it correlates with other properties of the papers may suggest answers. To quantify the data, I use a words-in-mean-paper-index (WIMPI), whose charm is that it readily permits comparisons among disciplines, decades and nations. Investigations of the subject can easily be performed by anyone with access to a large library and a strong index finger (at least the latter should probably be your own).

A preliminary investigation of one English-language journal each of physics, mathematics and chemistry from the UK, the USA and Japan (see the figure legend) shows that the trend spotted by Abt and Harris is very widespread. For every

journal examined, WIMPI was larger in 1980–83 than in 1950. Increases ranged from 13 to 115 per cent, with an unweighted average of 64 per cent. This drops to 43 per cent, however, if we set the Japanese baseline year at 1955, by which time the effects of the immediate post-war woodpulp shortage had largely disappeared.

The 13 per cent minimum value belongs to *Monthly Notices of the Royal Astronomical Society* and the 115 per cent maximum to *Journal of the Mathematical Society of Japan*. After renormalizing the Japanese data, *Journal of the Chemical Society London*, *Journal of the American Chemical Society* and the American astronomical journals tie at 82–85 per cent for the largest increases in WIMPI since World War II. Disciplinary and national averages for the per cent increases in words per paper during 1950–80 are: physics, 27; mathematics, 77; astronomy, 62; chemistry, 93; UK, 45; USA, 65; Japan, 85 (but only 20 per cent since 1955). Every reader can surely find something in these numbers to support his most cherished preconceptions.

Among the letter sections and journals (most of which are not more than 20 years old in their present formats), 10 year increases in WIMPI range from 5 per cent (*J. Am. chem. Soc.*) to 38 per cent (*Bulletin of the Chemical Society of Japan*) and 20 year increases from 40 per cent (*Bull. chem. Soc. Japan*) to 170 per cent (*Journal of Physics*). The average values are 26 per cent over the past 10 years and 76 per cent over the past 20. Some of the changes closely parallel changes in editorial policy; for instance, the mean paper length in *Physical Letters B* rose from 3.6 pages in 1973, when

the rule was "maximum length not to exceed three printed pages", to 4.6 pages in 1983 when it read "should normally not exceed".

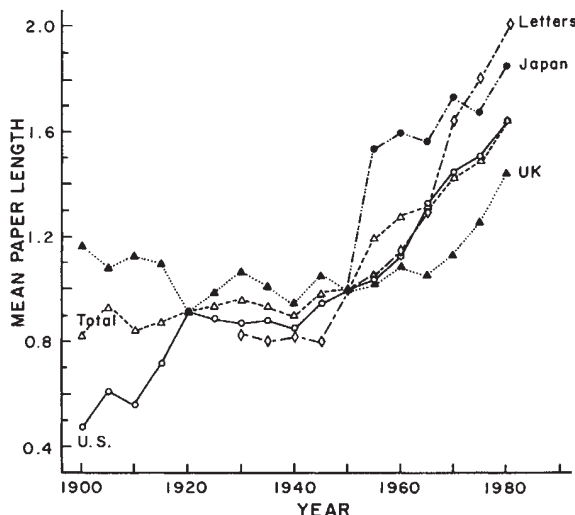
The net result is a 1960–80 average increase of 76 per cent in WIMPI for the letters publications, compared to 29 per cent for their associated main journals. Such a trend persisting over half a century would give us 12,000 word 'letters', equivalent to about 12 pages of *Nature* (whose authors are currently expected to review even rather broad topics in no more than 6). The high prestige associated with getting one's work into many of the letter publications seemingly encourages authors to push constantly at the length limits so as to squeeze in contributions that should really be full papers.

The complete range of data shows several other trends, strongly suggestive of causality. UK paper lengths shrank (though not as much as total numbers of papers published) during the First World War and the Depression and rose slightly during World War II (while paper numbers, of course, dropped enormously). American paper lengths changed little during these periods. The reader will immediately think of a variety of explanations in terms of the kinds of people available to do scientific research and the resources available to them. For *Physical Review* and *Proceedings of the Physical Society* (= *Journal of Physics*) the 1980 WIMPIs have only just climbed back to the pre-war peaks reached in 1920 and 1930 respectively. *Annals of Mathematics* is unique in showing a continuous nearly-linear rise in WIMPI ever since the turn of the century. Its 1980 papers are, on average, 3.3 times the length of its 1900 ones, reflecting an increasing determination on the part of the editors to accept only papers that present a major new idea in full detail (Rector, D.L., personal communication).

The main trends — universal post-war WIMPI creep most heavily concentrated in letter publications, correlations with world economic and political events, responses to editorial policy, and differences among disciplines and nations in (perhaps) expected directions — are all strongly suggestive of an effect largely driven by forces external to the kind of scientific research being reported. This, in turn, provides some hope of moderating the current exponential literature growth. Unfortunately, the sociology of publishing is such that each author's doing what he perceives as best for himself is unlikely to lead to the best possible result for the scientific community as a whole. Perhaps the time has come for some sort of Social Contract among authors, editors, referees, readers and sponsoring agencies. □

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Changes in WIMPI, normalized to 1950 = 1.00. The journals represented are: for the UK, *J. chem. Soc.* (1900–82), *Proc. Lond. math. Soc.* (1903–81), *Proc. Phys. Soc. Lond.* (became *J. Phys.* 1921–83) and *Mon. Not. R. astr. Soc.* (1940–82); for the USA, *Ann. Math.* (1900–80), *Phys. Rev.* (1913–83), *J. Am. chem. Soc.* (1920–80) and the American astronomical journals studied by Abt (1910–80); and for Japan, *Bull. chem. Soc. Japan*, *J. Math. Soc. Japan*, *Prog. theor. Phys.* and *Publs astr. Soc. Japan* (all 1950–80). The letters average includes the corresponding short-communications sections and journals plus *Phys. Lett. B*, published in Europe. Data for the earlier years are generally averages (for example, 1910 = 1908–12) and each point represents at least 300 papers.



Geophysics

The nightside magnetosphere

from S. W. H. Cowley

THE first sets of data collected by the International Sun Earth Explorer-3 (ISEE-3) spacecraft during its passage through the nightside magnetosphere, and recently published, are yielding results of major importance for magnetospheric physics¹⁻⁹. They provide the first direct evidence for the long-held belief that the Earth's magnetosphere has a very asymmetric shape and new insight into the behaviour of the magnetospheric tail during substorms.

All we know about the Earth's magnetosphere, the cavity in the solar wind plasma formed by the Earth's magnetic field, points to the fact that it should have a very asymmetric shape, but direct evidence has, until now, been lacking. The field on the sunward side is compressed by the ~ 400 km s⁻¹ solar wind flow, and extends to distances of only ~ 10 Earth radii ($1 R_E \sim 6,400$ km). On the nightside, however, coupling between magnetospheric flux tubes and the solar wind stretches the magnetosphere into a long cylindrical magnetic tail consisting basically of two bundles of oppositely-directed magnetic flux connected to the Earth's polar regions (Fig. 1a). As long ago as 1965 Dungey showed, by considering the motions of the polar ionosphere, that the tail should extend $\sim 1,000 R_E$ downstream of the Earth¹⁰.

Until recently, detailed spacecraft observations of the tail were available only out to distances of a few tens of Earth radii and relatively little was known about the properties of the nightside magnetosphere beyond the lunar orbit ($\sim 60 R_E$). In mid-1982, however, the ISEE-3 spacecraft was moved from its orbit about the dayside Lagrangean point $234 R_E$ sunward of the Earth, where it had been monitoring solar wind particles and field since its launch in 1978, and, by means of novel lunar gravity-assisted orbit manoeuvres, sent on a series of passes through uncharted nightside territory extending $\sim 235 R_E$ from the Earth.

The first major result to emerge from the new data is that although the distant tail retains a well-ordered magnetic structure similar to that found near the Earth^{2,4}, the character of its plasma populations is distinctly different. In the region near the Earth, the magnetic lobes of the tail contain only a tenuous low-energy plasma, and between the lobes, during magnetically quiet conditions, there is a thick ($\sim 5 R_E$) region of hot (~ 5 keV) plasma, named the 'plasma sheet', which drifts slowly towards the Earth with average speeds of ~ 100 km s⁻¹ (Fig. 1a). At $\sim 200 R_E$, ISEE-3 made two discoveries: first, the tail lobes are often pervaded by plasma streaming tailward at speeds of ~ 200 km s⁻¹, but still having fairly low densities compared with,

for example, the density of the solar wind (~ 5 ions per cm³); and second, at the centre of the tail, between the lobes, are plasma jetting tailward at speeds of 500 – $1,000$ km s⁻¹ and tailward-flowing energetic particles^{1,3,6}. The first of these findings is rather unsurprising and can easily be explained by continual drift into the tail lobes of solar wind plasma gaining access along 'open' magnetic field lines at the tail boundary. The second finding is much more important, and provides the first insight into the location of the much-sought quiet-time tail magnetic neutral line. This line lies at the centre of the 'X' in the magnetic field shown in Fig. 1a, and forms the boundary between 'closed' field lines which flow earthward at the tail centre plane and 'disconnected' field lines which flow tailward (according to Dungey's 'open' model of the magnetosphere). The ISEE-3 flow measurements at the tail centre plane indicate that the quiet-time neutral line lies at a downtail distance of $\sim 100 R_E$ (ref. 8).

The second major result concerns the behaviour of the tail during substorms. These 'storms' occur a few times each day, last about 1 hour, and are associated with brilliant disturbed auroral displays that can be seen from Earth at high latitudes and

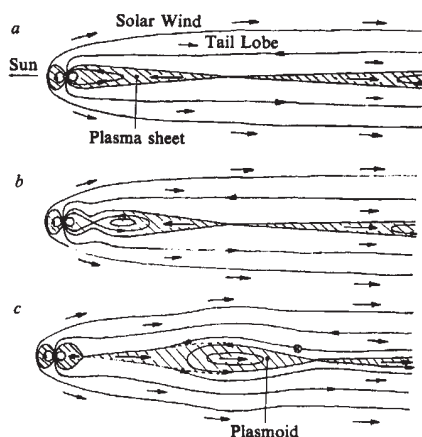


Fig. 1 Sketches of the Earth's magnetosphere in the noon-midnight meridian plane showing, approximately to scale, the large-scale reconfiguration which occurs during substorms (after ref. 5). Solid lines are magnetic field lines, short arrows indicate the direction of plasma flow and hatching the regions of hot accelerated plasma. *a*, Pre-substorm configuration with the tail neutral line at $\sim 100 R_E$ from Earth. At the beginning of the substorm a new neutral line forms in the near-Earth plasma sheet resulting in its rapid disconnection from the Earth. The resulting 'plasmoid' of closed flux loops flows downtail (*c*) followed by hot tailward-streaming plasma accelerated downstream of the new neutral line.

with magnetic disturbances caused by the flow of intense currents in the ionosphere and magnetosphere. The ISEE-3 data provide new information strongly supporting the view that substorms are closely linked with large-scale rearrangements of the configuration of plasmas and fields in the nightside tail (refs 5 and 9 and see Fig. 1b, c). At the onset of a substorm, a new neutral line forms in the near-Earth tail (Fig. 1b) at a point where magnetic reconnection is sufficiently rapid that, within minutes, a large fraction of the pre-substorm plasma sheet becomes disconnected from the Earth, forming a large (tens of Earth radii) 'plasmoid' of closed field loops which flows downtail at speeds of ~ 500 km s⁻¹ (Fig. 1c). The ISEE-3 spacecraft first detects substorms ~ 20 – 25 min after their onset when it becomes magnetically connected to the hot plasma accelerated downstream of the new neutral line (for example, at the cross in Fig. 1c) and observes electrons and high-energy ions moving quickly from the 'tail' of the tailward-flowing thermal plasma, along the newly 'disconnected' field lines. After a further 5–10 min, ISEE-3 is engulfed for 20 min by the hot plasma and disturbed magnetic field region of the plasmoid, before emerging into the hot substorm-accelerated tailward-streaming plasma itself. After ~ 40 min the new neutral line is believed to start moving tailward towards a position at $\sim 100 R_E$, and this signals the beginning of an ~ 40 min recovery period. Towards the end of this period, ISEE-3 usually leaves the tailward-streaming plasma and moves into the tail lobes, though it can remain in the plasma sheet for hours.

With the advent of Halley in 1986, it is interesting to note that similar 'disconnection' phenomena appear sometimes to occur in the plasma tails of comets. In the case of comets, however, the tail is not formed by magnetism intrinsic to the comet, as is the case for the Earth, but by solar wind field lines which are 'caught up' near the comet head in cometary plasma and which are then stretched out downstream to form an 'induced' magnetotail. ISEE-3 should provide the first observations *in situ* of these magnetotail phenomena as well. When it had completed its deep-tail passes, the spacecraft was targeted within ~ 120 km of the lunar surface on its last lunar interaction in December 1983, and received sufficient impulse to escape the Earth altogether and pass through the tail of comet Giacobini-Zinner on 11 September 1985. □

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Evolution

DNA map of the human lineage

from Jared Diamond

AMONG the primates, man is most closely related to the great apes, especially to the chimpanzee and gorilla. The fine details of branching patterns within the ape-human lineage have remained elusive, however. Recent molecular and chromosomal banding studies have failed to provide decisive new information because humans, chimpanzees and gorillas are too closely related on these criteria. Of the three possible evolutionary relationships of the three genera ($H-\bar{C}-\bar{G}$, $H-\bar{G}-\bar{C}$, $G-\bar{C}-\bar{H}$, where $H-\bar{C}-\bar{G}$ means that the gorilla line diverged from the common ancestor of humans and chimpanzees before the latter two lines diverged from each other), all have found recent advocates. There is also the question of absolute dating: when did each evolutionary branch-point occur? A paper by Charles Sibley and Jon Ahlquist of Yale University, which has just been published in the *Journal of Molecular Evolution* (20, 2; 1984), may provide the answers we have been waiting for.

An increasingly popular approach to examining our ancestry is by molecular clock methods. Early clock studies based on individual proteins or on fractions of the mitochondrial DNA suffered from the disadvantage that different fractions of the genome evolve at different rates, leading to discrepancies among results from different 'clocks'. The aim has therefore been to measure genetic distance based on the entire genome, which is possible by the single-copy DNA-DNA hybridization technique. In a massive survey of more than 1,000 bird species, Sibley and Ahlquist previously established by this method that the whole genome exhibits a uniform average rate of evolution among bird species (see *Nature, News and Views* 305, 17; 1983, for a brief synopsis and references). Now they present the long-awaited results of a study of human evolution by the same method.

Sibley and Ahlquist were able to determine the entire matrix of DNA hybrids for humans, the four ape lineages (chimpanzee, gorilla, orangutan and gibbon) and Old World monkeys, including the chimpanzee itself (*Pan troglodytes*), the pygmy chimpanzee (*Pan paniscus*), two gibbon species and five monkey species. The experimental accuracy of the results and the agreement between reciprocal DNA-DNA hybrids are excellent.

The results show that man and chimpanzee are slightly more closely related to each other than either is to the gorilla, indicating that the gorilla lineage was the first to diverge. As a quantitative expression of genetic distance, Sibley and Ahlquist use a measure of DNA-DNA hybrid dissociation temperature named

the $\Delta T_{50} H$ statistic, which is also proportional to elapsed time since an evolutionary branch-point. For man and chimpanzee $\Delta T_{50} H$ proves to be 1.9, which implies a genetic distance greater than that between the chimpanzee and pygmy chimpanzee (0.7); similar to that between grey-crowned and white-browed babblers (1.5) and between Cory's and sooty shearwaters (1.8); slightly less than the distance of the gorilla from chimpanzee and man (2.1–2.4), or between two species of gibbons (2.2); less than the distance between red-eyed and white-eyed vireos (2.9) and between chimpanzee and orangutan (3.7); and much less than the distance between two of Britain's most familiar warblers, the garden warbler and chiffchaff (7.5).

$\Delta T_{50} H$ values can be calibrated to absolute dates if the ages of some DNA-dated evolutionary branch-points can be established independently. Sibley and Ahlquist establish such a calibration based on fossil evidence that orangutans branched from the human-chimpanzee-gorilla lineage between 13 and 16 Myr ago and supported by more extensive avian data. Measured $\Delta T_{50} H$ values can then be translated into the following times of divergence (millions of years ago) from the chimpanzee lineage: Old World monkeys, 27–33 Myr ago; gibbons, 18–22; gorilla, 8–10; humans, 6.3–7.7; pygmy

chimpanzee, 2.4–3.0. Two gibbon species diverged from each other 7.7–9.5 Myr ago, around the time that the gorilla diverged from the chimpanzee-human line.

One of the tenets of the taxonomic philosophy called cladistics is that classifications should be based on times of divergence rather than on subjective evaluation of similarity. $\Delta T_{50} H$ values thus provide the basis for a cladistic classification. Sibley and Ahlquist note that their values would thereby lead to changes in hominoid classification, but they prefer to postpone all such discussion until more values are available. One implication of personal interest to *Nature* readers deserves mention however. Among birds, species with $\Delta T_{50} H$ values up to about 4 are generally sufficiently similar that traditional taxonomists assign them to the same genus. Anthropocentrism may prevent even the most devout cladist on Earth from drawing the inevitable bitter consequence, but a cladist from outer space would surely consider the chimpanzee, pygmy chimpanzee, man and gorilla as congeners. What remains to be determined is which genus name has priority by the laws of zoological nomenclature. Will our children leave our scientific name intact while recognizing an expanded genus *Homo* with the four extant species *Homo gorilla*, *Homo paniscus*, *Homo sapiens* and *Homo troglodytes*? Or will they reclassify us as *Pan sapiens*, the wise chimpanzee? □

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Auditory physiology

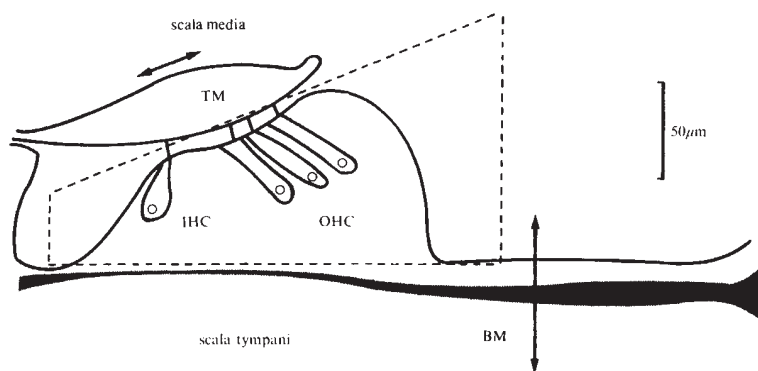
Splitting hairs over hearing?

from Jonathan Ashmore

WE really ought to know by now how the ear works. The essential event in mammalian hearing occurs in the cochlea, a coiled fluid-filled compartment of the inner ear. In outline, sound sets up a vibration pattern in the basilar membrane which forms a dividing partition of the cochlea (see the figure)¹. This design is used by mammals to hear low as well as, what are to us, ultrasonic frequencies. The details of the mechanism are only beginning to come clear. It is now known that hearing is an active process — the ability of the cochlea to discriminate frequencies is energy dependent. It is also apparent that, normally, the amplitude of the basilar membrane motion saturates at quite low sound levels, indicating that the movement involves nonlinear elements. What further distinguishes cochlear mechanics from an exercise in three-dimensional fluid dynamics is the presence in the cochlea of hair cells, neural elements possessing a bundle of rod-like stereocilia

which are deflected as the basilar membrane moves. Although hair cells are usually considered to be sensory cells, Flock and Streltsov address, in this week's *Nature*² (p. 579), the question of whether the hair cells themselves have mechanical properties which contribute to the tuning mechanisms in the cochlear partition, and in doing so present the first direct data on individually-manipulated mammalian hair cells.

Frequency selectivity, the ability of the cochlea to discriminate frequency components in a complex sound, is believed to depend on the mechanics of the basilar membrane and interactions within the cochlear partition³. The position of the peak basilar membrane vibration depends on the sound frequency, and is much more localized than was once thought^{4,5}. Early reports that mechanical and neural tuning differed may now have a variety of technical explanations. The difficulty of cochlear experimentation may indicate



Cross-section of the cochlear partition at a mid cochlea position. Scala media contains high-potassium fluid; scala tympani contains normal extracellular fluid. Motion (arrowed) of the basilar membrane (BM) causes a shear displacement of the tectorial membrane (TM) against the hair cell stereocilia. Inner (IHC) and outer (OHC) hair cell rows shown only. The dotted line delineates the part of the organ of Corti used by Flock and Strelioff. (After ref.5.)

that sharp tuning depends on a complex interaction between components of the cochlear partition which is easily and irreversibly disturbed.

The neural components most likely to be involved in improving frequency selectivity are the outer hair cells. They are well placed to modify the movement of the tectorial membrane⁶ and, three times as numerous as the inner hair cells, they also form the main target of the centrifugal pathways to the cochlea. Evidence that hair cells contain actin in their stereocilia⁷, as well as a whole array of proteins normally associated with contractility⁸, supports earlier suggestions that the outer hair cells are end organs rather than sensory cells, with the implication that they have a function in cochlear mechanics. It is not clear, however, whether the cells themselves are the active elements, injecting energy into the cochlear partition on a cycle-by-cycle basis, as theoretical models may require⁹, or whether their role is to modulate the coupling of the basilar membrane to some as yet undefined energy source.

Flock and Strelioff now suggest that the hair bundles of both the inner and outer hair cells in the isolated guinea pig organ of Corti cannot be treated as uniform coupling elements in the partition. Thus, hair bundles resist displacement asymmetrically, being more resistant when deflected in the functional excitatory direction. The physiologically very large (1 μ m) static deflections applied would correspond to a sound level in excess of 100 decibels. Although the results must be viewed with a degree of caution because of some uncertainty about the coupling of the micromanipulating probe to the hair bundle due to the small scales involved, an asymmetry of this type in the cells *in vivo* certainly would have consequences for the mechanics of the basilar membrane.

The results further indicate that the stiffness of the bundle is graded with cochlear position, the cells at the apical (low-frequency) end being about five times less stiff than those in the basal cochlea. This is not a result of reduced lever advantage, although the stereocilia do

become shorter towards the basal end. The finding is a surprise, despite hints of something similar in one type of reptilian ear where the frequencies to which the hair cells respond depends on dimensions of the stereocilia¹⁰. In most models of cochlear mechanics, it is the elasticity of the cochlear partition that is varied to produce the observed range of frequencies¹¹. A contribution to the elasticity by the hair cells might reduce the discrepancy between theoretical models and experimental data.

What measurements of this kind cannot

clarify is the nature of the physiologically labile component of the cochlear partition which ultimately results in the observed frequency selectivity. The paradox is, of course, that cell isolation is precisely the condition under which the hair cell mechanisms relevant to tuning may well be absent, since the effect of *in vitro* isolation is to abolish the ionic difference between apical and basal portions of the cell as well as the 120 mV potential difference that exists across the membrane bearing the sensory hair bundle. Hair cells may be more robust than previously thought, but we need to know more about the static and the dynamic properties of the cells under conditions approaching those normally found in the cochlea. □

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Holocene penguin extinction

from Colin Harrison

FROM bird bones found in a thirteenth-century midden on Hunter Island, off Tasmania, G. F. Tets and S. O'Connor have identified an extinct genus and species of penguin, *Tasidyptes hunteri* (*Records of the Queen Victoria Museum, Launceston* **81**; 1983). Apparently related to other penguins now found around New Zealand, and about the size of the recent Rockhopper Penguin, it provides the first evidence of extinction of a penguin during the Holocene.

G. G. Simpson has remarked on the greater number of penguin species present in the Upper Tertiary than now. The last of these lost forms seems to have disappeared with the changes in climate that ushered in the Pleistocene, a time at which we also lost the huge Bony-toothed Seabirds and, incidentally, the North Atlantic Albatross. Subsequent to this, the penguins had appeared to be a stable, if relict, group of species, too isolated to be seriously harmed by man.

Finds of extinct birds on various islands of the Indian, Pacific and Antarctic Oceans have begun to change our picture of the avifauna of these regions. We now know that the present birdlife of New Zealand, Madagascar, the Mascarenes and the Hawaiian Islands represents the relics of larger faunas devastated by man in historic times.

Other very recent discoveries have included a number of as yet undescribed species on New Caledonia together with a huge cassowary-sized megapode possibly related to the very large Pleistocene megapodes of eastern Australia. The period of these early widespread extinctions apparently attributable to man seems mainly to have been the latter part of the last millenium and the early part of the present one, before the European incursion into these regions.

The new evidence does not change our ideas on the general distribution of taxa at family or subfamily levels, but it does indicate the existence, in the past, of a greater and more striking diversity of forms than we had previously assumed. It also underlines the need for caution in erecting hypotheses based on the number and variety of bird species now present in such places. Finally, even after allowance has been made for habitat-changes that may have accompanied these extinctions, the absence of the lost species must have affected the potential distribution and populations of those remaining, and may help to explain successful and rapid colonizations by some species more recently introduced. □

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Particle physics

Higgs discovery at DESY?

from Robert Walgate

THE Higgs particle — a vibration of the 'aether' with which modern unified theories of the forces of nature fill previously 'empty' space — may have been discovered in Hamburg. But there is controversy in the six-nation 73-physics team working at the Deutsches Elektronen Synchrotron (DESY) on the American 'crystal ball' detector as to whether the evidence is yet firm enough to announce. A member of the team, now at DESY's counterpart and rival laboratory, the Stanford Linear Accelerator Center (SLAC) in California, leaked the result to the press, leaving DESY in the somewhat embarrassing position of having SLAC announce one of its own results.

Nevertheless, say DESY, there is evidence for a particle of 8.3 GeV (about eight times the proton's mass) which *may* be the Higgs, or aether particle. However the particle, dubbed 'zeta' by SLAC (who can claim an interest as they built the crystal ball), is produced about 20 times more rapidly than theory would predict. It is found in the decay of the ψ , a particle consisting of a heavy 'bottom' quark bound to its antiparticle.

The group now has 100,000 examples of ψ decaying into a photon and something else, and in just over 100 cases — a tiny fraction — they say this something else is a very narrow (long-lived) state of mass 8.3 GeV, a place where little else than a Higgs could live.

The state appears as a spike in a histogram, about 5 standard deviations above background, and has resisted several attempts to remove it by alterations in the very complex data analysis programmes. In particular, the ψ -prime, a close excited state of the ψ , does not show the peak at all. If it were a data-processing effect, it should show up in the ψ -prime as well.

However this leads to another difficulty — for if it were Higgs' particle, the ψ -prime should decay to it just as does the ψ , and so also show the peak.

Nevertheless, a DESY spokesman last week described the ψ peak as "beautiful". But he also explained that the crystal ball is here working in a very dirty region, highly contaminated by photons from π -zero decays, so the computers have to work overtime to extract the tiny signal from a mass of noise.

Thus, with these and other problems, DESY thought it politic to wait for a few weeks — when the crystal ball group will take data on another 200,000 ψ decays and be able to check their result — before announcing that the Higgs, or even the zeta, was a reality.

As to how the Higgs may be considered a vibration of the aether, this goes back to

the theory of spontaneous symmetry breaking, which is needed in the Salam-Weinberg-Glashow (SWG) unification of the weak and electromagnetic forces. This and most other modern unified field theories require the vacuum to be full of a transparent, massless, but nonetheless real stuff (hence the analogy with the 'aether'). This stuff has two effects: first, it causes different fields of force within a unified field theory to propagate differently (for example, retarding the weak-force field in the SWG theory). This is the 'spontaneous

symmetry-breaking' effect. Then second, the stuff can itself vibrate, sending density waves — like sound waves — through the 'vacuum'. Since in quantum theory, waves and particles are aspects of the same reality, the 'sound' waves may be detected as particles. These are the Higgs particles. Their detection would be an important boost to the unified field theories, because it would prove — and probe — the presence of the vacuum-filling 'stuff' on which the theories depend. Unfortunately, the theories give little clue to the elasticity of the vacuum — and consequently to the mass of the corresponding Higgs particle. But a current lower limit is set at 7 GeV, just below the mass of the zeta. □

Robert Walgate is Chief European Correspondent for Nature.

Origins of life

Bounds to the amplification of biomolecular handedness

from Stephen Mason

THEORIES accounting for the dominance of the L-amino acids and the D-sugars in the biochemistry of living organisms centre upon mechanisms for the selection of enantiomers of one hand from the heterochiral mixture of a racemic substrate, and for the propagation of optically-pure homochiral products. Proceeding directly to a model for control of molecular turnover and propagation in the cell, Joyce *et al.*¹ (see p. 602) have investigated the template-directed replication of a polynucleotide from the individual enantiomers and from a racemic mixture of the corresponding monomers. Their results have important implications for theories of the origin of living systems.

Using poly-D-ribocytidylic acid [poly(C_D)] as the template catalyst, Joyce *et al.* find that oligomerization of D-guanosine 5'-phospho-2-methylimidazole (D-2-MeImpG) is highly efficient, giving specifically all 3'-5'-linked oligo(G_D)_n ranging in length from the dimer ($n = 2$) to $n = 20$. The poly(C_D) template-directed oligomerization of L-2-MeImpG is far less efficient, giving a mixture of three dimers as the major product, as occurs in the corresponding uncatalysed reaction. The minor trimeric and tetrameric products have an increased yield in the poly(C_D)-directed oligomerization of the L-isomer, however, indicating the persistence of a template effect.

In the template-directed reactions of the racemic DL-mixture of 2-MeImpG with the poly(C_D) catalyst, the L-isomer substantially reduces the stereospecificity and chain-propagation of the oligomerization of the D-isomer. Thus in these conditions, each n -fold oligomer product is now a mixture of up to four

isomers, and the more extended chain lengths ($n > 10$) are no longer detected. The results of other poly(C_D)-directed reactions, using ¹⁴C-labelled D-2-MeImpG, support the inference that, once a L-monomer has been added to either end of a growing oligomer, further growth is inhibited. Thus, the probable location of the L-guanosine residues in a DL-mixed oligomer is either at the 5' terminus or the 2'(3') terminus, or at both positions. The four isomers of a given n -fold DL-oligomer correspond to the terminal-residue chiralities L-L, L-D, D-L and D-D.

Poly(C_D)-directed oligomerization of D-2-MeImpG is not inhibited by the activated mononucleotides of adenosine, cytidine or uridine. The inhibition is particular to the enantiomer L-2-MeImpG, and derives from the specific Watson-Crick base-pairing of cytidine with guanine. The D- or L-handedness of the ribose sugar in 2-MeImpG governs the conformation of the bound monomer around the glycosidic bond and the orientation of the sugar-phosphate unit relative to the poly(C_D) template. These factors favour the incorporation of D-2-MeImpG at the 2'(3') terminus of a growing oligomer, propagating antiparallel to the poly(C_D) template. They cannot, however, prevent the misincorporation of L-2-MeImpG under the impetus of specific cytidine-guanine base-pairing and, once in place, the L-guanosine residue limits the further addition of either enantiomeric monomer.

Some 25 years ago, it was found that the base-catalysed polymerization of the N -carboxyanhydrides of the L- α -amino acids is inhibited by the corresponding derivative of the D-enantiomer^{2,3}. Under comparable conditions, the rate of poly-

merization of either the L- or the D-monomer is 17 times faster than that of the DL-racemic mixture and 5 times more polymerization is attained². The propagation rate of a L-polypeptide in the α -helix conformation, with a L-monomer supply, is similarly reduced by the addition of the D-monomer and the extension of the helical secondary structure is no longer regular³.

The prebiotic implications of the enantiomeric cross-inhibition observed in these early studies of peptide polymerization were not far-reaching because the polynucleotides governing protein manufacture in the cell were expected to ensure the optical purity of the amino acid substrates selected. By contrast, the novel demonstration by Joyce *et al.* that the laboratory replication of polynucleotides displays a similar and more complex enantiomeric cross-inhibition bears more fundamentally on the origin of biomolecular handedness, and perhaps has implications even for the mineral realm⁴. Joyce *et al.* conclude that, if the origin of living systems was founded upon the replication of polynucleotides, there must already have been either mechanisms designed to minimize the effects of enantiomeric cross-inhibition, or localized prebiotic fractionation and segregation processes for the enantiomers of racemic biomolecules.

An example of homochiral polymer formation that is not sensitive to enantiomeric cross-inhibition is the spontaneous optical resolution of a racemic mixture by crystallization. Optically-pure single crystals of L-glutamic acid grow from seeded solutions of the racemate just as well as from solutions of the L-enantiomer. Most of the 20 common α -amino acids making up the proteins in cells can be spontaneously resolved into optically-pure single crystals by the crystallization of a racemic solution, either of the amino acid itself, or of a simple derivative, such as a salt⁵.

Recently, a remarkable enantioselection and segregation of the D- and L-isomers of α -amino acids by occlusion into growing crystals of the α -polymorph of glycine was discovered⁶. It can be explained as follows. A crystal of the α -form of glycine possesses an inversion centre of symmetry and is itself not handed. The inversion centre relates two opposite faces of the plate-like crystal, one, the (010) face, occluding only the D-isomer of a chiral α -amino acid, and the other, the (0 $\bar{1}$ 0) face, taking up solely the L-enantiomer. The low density of the glycine crystal (1.16 g cm⁻³) allows some of the crystal plates to float on the surface of the mother liquor, leaving only one of the two selector-faces exposed to the aqueous feedstock of glycine and racemic α -amino acid. During subsequent growth, the floating crystal takes up, along with further glycine molecules, only one of the two optical isomers of the chiral amino acid, leaving an excess of the enantiomer in solution.

If the chiral amino acid is hydrophobic, the excess of the enantiomer remaining in solution determines which of the two selector-faces of a small floating glycine crystal is orientated towards the aqueous side of the air-water interface for further growth. An excess of the D-isomer in solution ensures the orientation that requires the occlusion of the L-enantiomer from the mother liquor by the floating crystal, thus enhancing the enantiomeric excess of the D-isomer in the aqueous phase. A D/L composition of leucine as near equality as 12/10 with a 50-fold larger amount of glycine, in water, gives an assembly of completely orientated floating glycine crystals which progressively take up the L-isomer and amplify the initially-minor enantiomeric excess of D-leucine⁶.

What remains to be explained is how the initial enantiomeric excess came into being.

An amplification process of a quite different order is required to produce the starting point of an ~ 10 per cent enantiomeric excess from the excess abundance of the L-enantiomer in a racemic mixture of an α -amino acid or a polypeptide in the α -helix or β -sheet conformation at the level of 10⁶ molecules per mole predicted naturally to occur on the basis of the parity-violating electroweak interaction⁷. □

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Photosynthesis

Control of protein turnover by photosynthetic electron transport

from John Bennett

FOR the past ten years much of the interest in protein synthesis of plant-cell chloroplasts has centred round the identity and turnover of a 32,000-molecular weight polypeptide component of the photosynthetic membranes (thylakoids). The intriguing feature of this protein is that while it is probably the most rapidly synthesized protein of mature chloroplasts¹, its steady-state level is very low, presumably as a result of equally rapid breakdown. An important clue to its identity came from the discovery that it is specifically labelled when thylakoids are incubated with a radioactive azido analogue of the herbicide atrazine². One of several herbicides known to inhibit electron transport between photosystem II and the plastoquinone pool, atrazine acts by displacing from the photosystem II complex the secondary electron acceptor, a plastoquinone named Q_B³. The 32,000-molecular weight protein is therefore regarded as plastoquinone-binding protein and has been named Q_B-binding protein. But why should it be subject to such rapid turnover? This question has now been examined by two groups, both of whom suggest that turnover is the result of damage inflicted on Q_B-protein by its participation in photosynthetic electron transport^{4,5}.

Mattoo *et al.*⁴ have investigated the kinetics of Q_B-protein turnover in the small aquatic plant *Spirodela oligorrhiza*. Each plant consists of a few small leaves (fronds) and tiny roots. On transfer from light to dark, green fronds remain green for months but newly developing fronds emerge essentially devoid of chlorophyll and with few thylakoids. Upon exposure to light and after a lag period of 20–24 h, the white

fronds begin rapidly to turn green, reaching the normal light-grown condition within 2–3 days. Mattoo *et al.* show that neither green fronds nor white fronds synthesize the Q_B-protein at a significant rate in darkness. On transfer to light, however, green fronds synthesize the protein within 1 h whereas white fronds require 48–72 h of illumination before they synthesize the Q_B-protein. Mattoo *et al.* conclude that massive synthesis of the protein requires both light and the physical presence of thylakoids on which the 33,500-molecular weight precursor of the Q_B-protein are synthesized and into which the mature 32,000-molecular weight protein is inserted. Furthermore, the rate of synthesis increases with increasing light intensity, a response which Mattoo *et al.* attribute to a requirement for ATP generated by photophosphorylation.

Breakdown of the Q_B-protein is also dependent on light intensity, being very slow in darkness but rapid at 6,000 lux (full sunlight is about 100,000 lux). Q_B-protein synthesized at 3,000 lux is degraded completely within 5 h at 6,000 lux. Breakdown of the protein is not inhibited by inhibitors of photophosphorylation but it is abolished by atrazine and by another herbicide, diuron, which binds to much the same site on thylakoids as does atrazine and also inhibits electron transport. Electron transport is therefore more effective than ATP in inducing Q_B-protein breakdown and Mattoo *et al.* postulate that the Q_B-protein becomes prone to degradation by a membrane-bound protease as a consequence of its role in photosynthetic electron transport. They note that in isolated thylakoids the protein undergoes a light-

dependent transformation that affects its susceptibility to added trypsin. They do not specify how damage is incurred but assume that atrazine and diuron prevent that damage by inhibiting electron transport.

Kyle *et al.*⁵, on the other hand, make a specific proposal concerning the connection between electron transport and breakdown of Q_B -protein. Working with the green alga *Chlamydomonas reinhardtii*, they demonstrate a correlation between photoinhibition of photosystem II activity and the rate of turnover of Q_B -protein. When leaves or chloroplasts of higher plants are exposed to supraoptimal light intensities, their photosynthetic activity decreases⁶. This phenomenon is referred to as photoinhibition and is associated with damage to photosystem II. Kyle *et al.* induce a version of photoinhibition in *C. reinhardtii* by exposing cells to a light intensity ten times higher than that encountered during growth. The primary site of photoinhibition is at the reducing side of photosystem II (between photosystem II and plastoquinone) and the degree of photoinhibition is correlated with a loss of binding sites for ^{14}C -atrazine, suggesting that molecules of Q_B -protein have become non-functional. Since this form of photoinhibition is exacerbated by conditions which promote reduction of the plastoquinone (PQ) pool (high light intensities and blocking of PQH_2 oxidation), Kyle *et al.* suggest that a high PQH_2/PQ ratio increases the likelihood that the Q_B site will be occupied by the quinone anion Q_B^{2-} . Should Q_B^{2-} interact with O_2 generated by photosystem II, highly reactive oxygen radicals would be produced and would selectively damage Q_B -protein by reacting with specific amino acid side chains. Thus, Kyle *et al.* regard the breakdown of Q_B -protein as part of the process whereby damaged protein is removed from thylakoids; conversely, the continued synthesis and insertion of Q_B -protein is essential to the process of repair. Ironically, herbicides such as atrazine and diuron reduce the light-induced damage partly by slowing the rate of plastoquinone reduction and partly by occupying the Q_B -binding site and preventing the binding of Q_B^{2-} .

Further studies on this system will clearly be of importance for understanding how plants tolerate high light intensities. Of particular interest is the possibility that the synthesis of new Q_B -protein molecules is somehow regulated by the removal of damaged molecules. □

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Astronomy

IRAS circulars 14 and 15

Circular 14 contains 24 IRAS sources in a southern field between right ascension (RA) 10 and 14 h and between declination (Dec) -40 and -20 deg. All sources shown here are associated with galaxies on the ESO/SRC skyplates; all have larger infrared than visual luminosities. Selection by T. de Jong, University of Amsterdam.

Circular 15 contains a selection of infrared bright northern galaxies with radial velocities larger than $1,000 \text{ km s}^{-1}$; they are therefore sufficiently far away that the point source extraction algorithm will estimate the integrated fluxes correctly.

The source name consists of four parts: the letters IRAS to indicate the origin; RA in hours and minutes, seconds omitted; Dec in decimal degrees, multiplied by 10 and then truncated (i.e. 32 deg 42.3 min $\rightarrow$ +327); an appendix starting with 'P' and followed by the number of the circular, this appendix stresses that the data are preliminary. Position is given at equinox 1950.0. Measurements for circular 14 were made between epochs 1983.1 and 1983.9. am, arc min.

Source IRAS	RS h min s	Dec deg am	Flux densities (Janskys)			
			12 μm	25 μm	60 μm	100 μm
1021-284P14	10 21 57	-28 28.5	<0.2	1.0	5.4	7.1
1027-395P14	10 27 20	-39 35.1	<0.4	0.4	4.1	8.1
1108-282P14	11 08 22	-28 13.7	<0.3	0.6	3.7	5.1
1116-397P14	11 16 36	-39 43.9	<0.2	<0.3	2.5	5.5
1135-325P14	11 35 40	-32 35.1	<0.2	0.3	3.6	6.4
1140-273P14	11 40 50	-27 19.3	<0.2	<0.3	3.0	6.0
1146-330P14	11 46 24	-33 04.0	<0.2	<0.2	1.1	2.5
1150-388P14	11 50 40	-38 51.2	0.6	2.4	39.0	55.0
1203-322P14	12 03 09	-32 16.2	<0.2	<0.2	3.2	7.9
1204-316P14	12 04 17	-31 40.3	0.3	0.8	8.1	15.0
1206-364P14	12 06 24	-36 25.5	<0.3	<0.5	3.1	6.2
1217-356P14	12 17 21	-35 41.1	<0.3	<0.3	2.5	5.2
1227-398P14	12 27 00	-39 50.8	<0.2	<0.2	3.1	3.4
1228-260P14	12 28 39	-26 00.7	<0.2	0.9	4.7	8.3
1242-201P14	12 42 12	-20 09.0	<0.2	<0.4	3.1	7.4
1250-271P14	12 50 29	-27 11.5	<0.3	0.7	5.5	9.0
1255-294P14	12 55 02	-29 29.8	<0.4	1.0	7.0	10.2
1300-236P14	13 00 11	-23 39.2	<0.4	0.9	15.8	20.0
1304-335P14	13 04 22	-33 35.9	<0.3	0.6	5.3	9.2
1318-345P14	13 18 05	-34 34.6	<0.2	0.4	3.0	5.2
1318-314P14	13 18 07	-31 28.7	<0.2	<0.2	1.7	3.7
1319-394P14	13 19 45	-39 28.4	<0.2	0.5	2.9	3.5
1328-324P14	13 28 35	-32 29.2	<0.2	<0.3	1.8	3.6
1345-299P14	13 45 29	-29 57.0	<0.2	<0.3	2.5	3.6
0007+286P15	00 07 19	+25 38.8	0.5	1.2	10.2	19.6
0610+783P15	06 10 40	+78 22.5	6.5	18.5	171	260
0705+188P15	07 05 25	+18 51.6	0.6	2.2	22	40
0710+858P15	07 10 16	+85 50.9	0.6	1.2	12.9	36
0733+353P15	07 33 40	+35 21.2	0.5	1.0	9.7	16.5
0835+259P15	08 35 25	+25 55.8	<0.3	1.9	28	35
0842+742P15	08 42 33	+74 16.9	0.8	2.5	18.2	33
0910+403P15	09 10 54	+40 19.2	0.6	1.6	9.7	16.5
0914+422P15	09 14 10	+42 12.5	0.8	3.3	26	35
0939+320P15	09 39 55	+32 04.6	0.7	1.4	13.3	29
0951+018P15	09 51 06	+01 48.9	0.5	1.1	10.7	25
0958+559P15	09 58 35	+55 55.3	1.4	2.2	49	110
1012+736P15	10 12 39	+73 39.0	0.6	0.7	7.4	34
1013+213P15	10 13 48	+21 22.4	0.6	1.1	10.3	22
1020+201P15	10 20 47	+20 07.1	0.7	1.9	8.9	21
1035+537P15	10 35 40	+53 45.9	1.4	5.0	38	52
1049+232P15	10 49 53	+23 12.0	0.7	1.4	13.0	25
1100+282P15	11 00 27	+28 14.5	1.1	4.0	21	41
1120+168P15	11 20 17	+16 51.8	0.6	0.9	8.4	24
1124+571P15	11 24 43	+57 09.1	1.1	1.6	15.8	37
1146+489P15	11 46 01	+48 59.3	0.9	1.2	14.9	43
1211+548P15	12 11 42	+54 48.1	1.0	4.7	27	32
1308+373P15	13 08 37	+37 19.5	0.8	1.2	21	73
1330+630P15	13 30 27	+63 01.3	0.3	1.0	7.9	17.5
1359+595P15	13 59 09	+59 34.2	0.6	1.8	11.7	25
1430+581P15	14 30 38	+58 08.3	0.7	0.9	9.2	32
1534+167P15	15 34 14	+16 46.2	0.7	0.9	9.6	27
1809+149P15	18 09 35	+14 58.0	1.0	1.8	16.3	35
1821+745P15	18 21 13	+74 32.2	0.8	1.1	11.4	39
2026+255P15	20 26 27	+25 33.9	0.6	1.1	12.5	21
2300+086P15	23 00 45	+08 36.3	1.4	5.8	30	44
2302+120P15	23 02 26	+12 03.1	0.8	3.5	13.5	31
2312+042P15	23 12 11	+04 15.6	0.9	1.7	21	50
2317+169P15	23 17 60	+16 57.1	0.6	1.1	10.1	24

Developmental biology of a neural cell adhesion molecule

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A membrane glycoprotein isolated from embryonic brain has been shown to be a ligand in the formation of cell-cell bonds. The molecule, called neural cell adhesion molecule (N-CAM), participates in several aspects of neural development, including the formation of plexiform layers, neurite fasciculation and nerve-muscle interactions. Experiments in vivo indicate that N-CAM-mediated adhesion is essential to the orderly assembly of the visual system.

DEVELOPMENT of a nervous system is one of the most complex examples of morphogenesis in that it involves both the formation of intricate tissue structures and their precise interconnection. Of the many cellular events that contribute to this process, cell-cell recognition has received particular attention because of its potential for establishing specific patterns and interactions. From the experimental point of view, the major problem has been to devise an approach that will reveal not only the molecular basis of this recognition but also the mechanisms by which it contributes to tissue formation.

This review describes a membrane glycoprotein that is directly involved in adhesion between cells that express this molecule. Because it was first isolated from retina and brain, the name neural cell adhesion molecule or N-CAM has been adopted^{1,2}. However, as described below, the molecule is also expressed by some non-neural cells. Detailed reviews on the structure and properties of N-CAM have been published recently^{3,4}; the present description is intended to provide only a brief account of the isolation and biochemical studies of the molecule, and focuses primarily on the significance of N-CAM-mediated cell adhesion in the development of the nervous system.

In 1939, Holtfreter reported that mixtures of cells from different embryonic tissues would separate and develop structures characteristic of the parent tissues. He concluded that this *in vivo* morphogenesis required self organization of cells according to specific cell-surface affinities⁵. Several attempts were made to define the nature of the interactions involved in this phenomenon⁶⁻⁸, but only when short-term quantitative assays were developed⁹⁻¹¹ did it become feasible to test directly for 'recognition' substances.

The strategy used to isolate and study N-CAM is based on a variety of immunochemical techniques (Fig. 1). The antibody-neutralization assay used to detect N-CAM¹² was first applied to slime mould adhesion¹³, and has been used with considerable success in several other studies¹⁴⁻¹⁸. Such assays can in principle be used to investigate any phenomenon that is inhibitable by an antibody. They are particularly effective when used to obtain a small amount of antigen for production of monoclonal antibodies, which in turn are used to affinity-purify larger quantities of antigen¹⁸.

Biochemistry of N-CAM

Figure 2a gives a tentative structure¹⁹ for N-CAM. The model represents conclusions that N-CAM is an integral membrane glycoprotein with a single polypeptide chain, and that the molecule is divided into three domains, an amino-terminal region that forms a binding site (as defined below), a central region that contains the bulk of the carbohydrate, and a carboxyl-terminal region associated with the cell membrane. Two of the most striking chemical properties of N-CAM are the high and variable sialic acid content of its carbohydrate moiety¹⁸ (Fig. 2b) and its specific affinity for cell surfaces²⁰ (Fig. 2c).

The carbohydrate heterogeneity includes not only variations among molecules from the same tissue, but also between N-CAMs from different regions of the nervous system or from embryonic and adult animals²¹.

Several observations suggest that N-CAM can serve as a ligand in the formation of cell-cell bonds^{20,22}. The initial evidence was that specific antibodies against N-CAM are potent inhibitors of both N-CAM-cell binding and neural cell-cell adhesion (Fig. 2c, bottom). Subsequently, it was shown that artificial membrane vesicles containing only lipids and N-CAM can bind to the surface of neural cells (Fig. 2c, top) and that intact retinal cells and the artificial N-CAM vesicles display the same specificity in their binding to a variety of cell types. Although the mechanism of adhesion has not been established, there is substantial evidence that the binding process involves a direct interaction between N-CAM molecules²⁰. For example, binding that is inhibited by anti-N-CAM Fab has only been observed between two cells, both of which have N-CAM on their surface, and coating of only one of these cells with the Fab is sufficient to block its binding to the other cell. Furthermore, N-CAM vesicles themselves aggregate, suggesting that the purified molecule has homophilic binding properties.

What is the relationship of the work reviewed here to other cell-cell adhesion systems that have been described? Although most adhesion-associated molecules have not been as well characterized in terms of binding activity or *in vivo* function, some general statements can be made. First, it is now well established that the cell-surface antigens D2 (ref. 23), BSP-2 (ref. 24) and 2241A6 (ref. 25), and the unique sialic acid-containing moiety identified by Finne²⁶ are closely related to or derived from N-CAM. On the other hand, N-CAM mediates a calcium-independent adhesion that is functionally and antigenically distinct from calcium-dependent cell adhesion systems²⁷⁻³¹ and molecules including L-CAM^{15,16,32} and uvomorulin³⁰. Recently, two neural cell-surface molecules, Ng-CAM (nerve-glial cell adhesion)³³ and the L1 antigen³⁴, have also been identified as components of calcium-independent adhesion systems. Although each represents a different gene product, immunological cross-reactivities have been reported which suggest that they share at least one antigenic determinant with N-CAM^{33,35}. It is possible, therefore, that the calcium-independent systems reflect a family of molecules with a common structural element³⁵. In addition, there is evidence that N-CAM and L1 co-exist on the same cell and could act in combination to augment the specificity of neural cell-cell adhesion³⁶.

Role of N-CAM in neural development

At first glance N-CAM would seem to be an unattractive basis for a cell recognition system in that it is expressed on a wide variety of cells and the molecule seems to have only a single binding specificity. To understand N-CAM's role in development, it is necessary to consider morphogenetic mechanisms

other than strict chemospecificity. These include the regulation of N-CAM expression^{1,37}, effects of the carbohydrate heterogeneity on binding function¹⁹ and the biological consequences of a homophilic adhesion mechanism.

N-CAM seems to be continuously expressed during the formation of both nerve and striated muscle tissues (Table 1), and has recently been identified on cultured astrocytes³⁸. In contrast, many tissues that are adjacent to developing nerve and muscle tissues do not express the molecule, and it is notable that smooth muscle, which has a less intimate relationship with nerve than does striated muscle, does not produce N-CAM during its development³⁷.

A striking aspect of N-CAM expression is the transient appearance or disappearance of the molecule at sites associated with dynamic reorganization of tissue structure. These areas include placodes, the notochord and precursors of the mesonephric tubule. Of particular interest is the loss of N-CAM from the surface of neural crest cells that are migrating between the somite and neural tube and its re-expression by those cells when they aggregate to form a dorsal root ganglion³⁷.

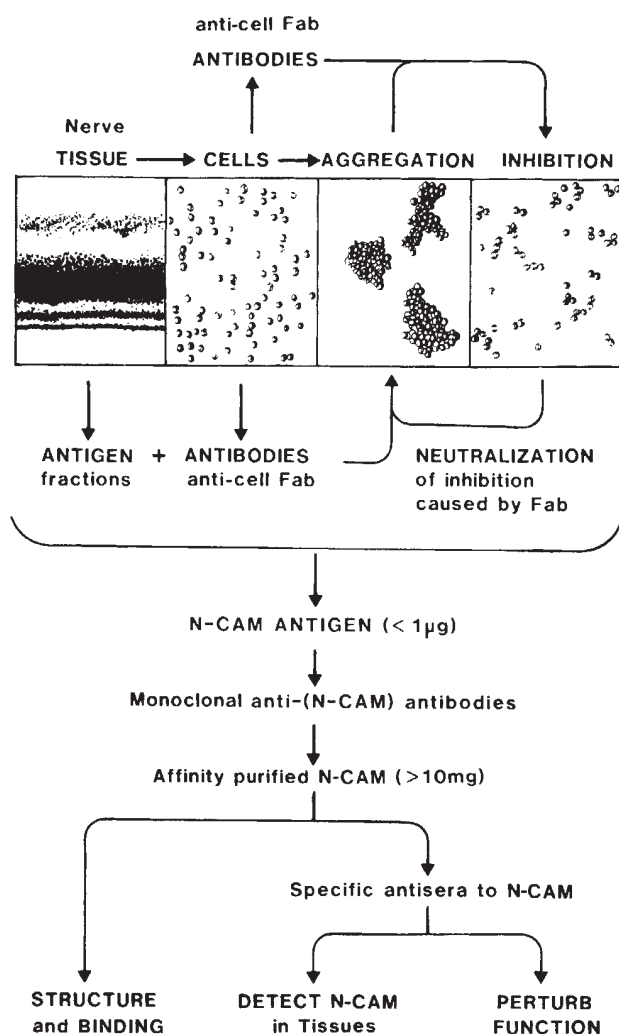


Fig. 1 Experimental approach used in the detection, purification and characterization of a cell adhesion molecule from nerve tissue. The adhesiveness of cells is determined by measuring the rate of decrease in particle number which accompanies their aggregation during a 20-min incubation. The assay for N-CAM is based on the ability of this molecule to neutralize specifically the adhesion-blocking activity of anti-nerve cell Fab. N-CAM is an abundant cell-surface molecule (~1% of the total cell-surface protein) and immunoaffinity chromatography procedures have allowed preparation of sufficient quantities of purified material for extensive biochemical studies.

Table 1 Expression of N-CAM during development³⁷

Continuous

Primitive neuroepithelium (neural plate, neural tube, brain rudiment)
Differentiated central and peripheral nerve cells
Developing skeletal muscle (somites, dermomyotome, myoblasts, myotubes)
Developing cardiac muscle (myocardium)

Transient

Neural crest (not expressed during migration)
Notochord
Placodes (lens, otic, pharyngeal)
Mesonephric primordium

The heterogeneity in N-CAM's sialic acid content influences its binding properties, as removal of this negatively charged sugar increases the rate of binding of N-CAM to cells by almost an order of magnitude¹⁹. Although the mechanism of this effect (for example, charge repulsion between two N-CAMs, alterations in conformation) is unknown, it seems to reflect a change in the affinity constant³⁹. Therefore, the potential exists that differences in both the amount^{1,37,39} and carbohydrate heterogeneity of N-CAM can produce a hierarchy of binding affinities which reflects both tissue origin and temporal regulation. As proposed originally by Steinberg⁷, such a differential affinity among cells can have a powerful influence on morphogenesis.

A homophilic binding mechanism could by itself produce specific patterns of cell association during development, for example in the release and subsequent reassociation of neural crest cells that migrate to form a spinal ganglion, through preservation of spatial relationships among neurites in a nerve bundle and by signalling to motoneuron axons that they have arrived at their target tissue. *In vitro* evidence for such nerve-nerve and nerve-muscle interactions is presented below.

Biology of N-CAM-mediated adhesion

The investigation of the biological function of N-CAM began with a series of *in vitro* experiments whose relative simplicity has allowed the identification of particular morphogenetic events that are influenced by this molecule (Fig. 3).

Formation of discrete cell and neurite layers. Culture of retinal cell aggregates produces patterns which resemble the differentiated tissue, in particular the concentration of neurites into regions free of cell somata⁴⁰. If the cultures contain anti-N-CAM Fab, this separation fails to occur, although the growth of neurites and other aspects of cell differentiation are not detectably altered²². Similarly, *in vitro* development of retinal tissue in the presence of the antibody⁴¹ does not produce plexiform layers free of cell bodies (Fig. 3a). Regional differences in the amount or form of retinal N-CAM might explain this sorting-out phenomenon, but have not as yet been detected. On the other hand, considering the physical differences between cell somata and their processes, a plausible explanation for the separation of cell bodies and neurites might be simply that membrane-membrane contact is maximized when the neurites are packed together in tight bundles.

Neurite fasciculation and branching. Other than plexiform layers, the most conspicuous associations among nerve processes are the fasciculated nerve tracts that connect different parts of the nervous system. The role of N-CAM in nerve fasciculation was examined *in vitro* using dorsal root ganglion explants (Fig. 3b)⁴². The outgrowth of neurites from these ganglia usually does not consist of separate fibres, but rather of thick bundles containing up to several hundred neurites. In the presence of anti-N-CAM Fab, the density and rate of neurite outgrowth are not obviously changed, but the diameter of the fascicles is markedly reduced. Bundles can still form, but rapidly split apart and exchange fibres with other fascicles.

The size and stability of fascicles also depend on the adhesiveness of the substrate to which the growth cones are attached.

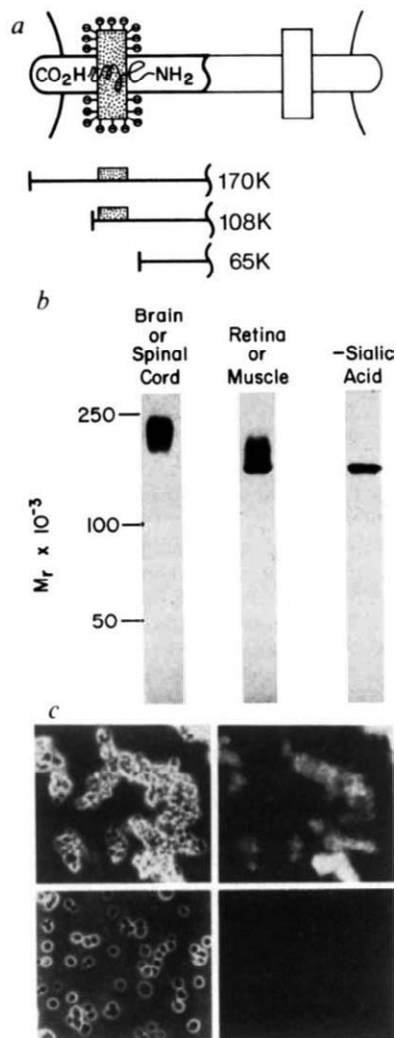


Fig. 2 Biochemical properties of N-CAM. **a**, Schematic representation of the N-CAM glycopeptide, showing a linear arrangement of an amino-terminal binding site, a central region with sialic acid-containing carbohydrate moieties (stippled areas) and a carboxyl-terminal domain which is associated with the cell membrane¹⁹. Although the carbohydrate is not located in the amino-terminal portion of the polypeptide, its sialic acid content has an important influence on the overall binding properties of the molecule (see text). Along with the size and properties of the intact molecule¹⁸, the key elements of this analysis are a 65,000-molecular weight (M_r , 65 K) fragment containing a binding site and little or no carbohydrate, a 108 K fragment released from cells by proteolysis which has both binding activity and a high carbohydrate content, and the demonstration that N-CAM and both fragments have the same amino-terminal amino acid sequence. A variety of evidence²⁰ suggests that cell-cell adhesion mediated by N-CAM involves homophilic binding between two N-CAM molecules, as depicted here in its most simplified form. **b**, Electrophoretic patterns reflecting heterogeneity in the sialic acid content of the molecule isolated from chick embryo retina or breast muscle (lane 1), and from embryonic brain or spinal cord (lane 2). Treatment of these N-CAMs with neuraminidase produces the same sharp band (lane 3), which has an apparent M_r in SDS of 140 K (as shown) and/or 170 K (refs 18, 19). The 140 K and 170 K polypeptides have many structural similarities¹⁹ and are probably derived from the same gene. **c**, Inhibition of retinal cell aggregation (top left compared with bottom) by anti-N-CAM Fab²² and binding of fluorescent artificial vesicles containing N-CAM to these cells²⁰ (top right). The vesicle-cell binding is also blocked by anti-N-CAM Fab (bottom right).

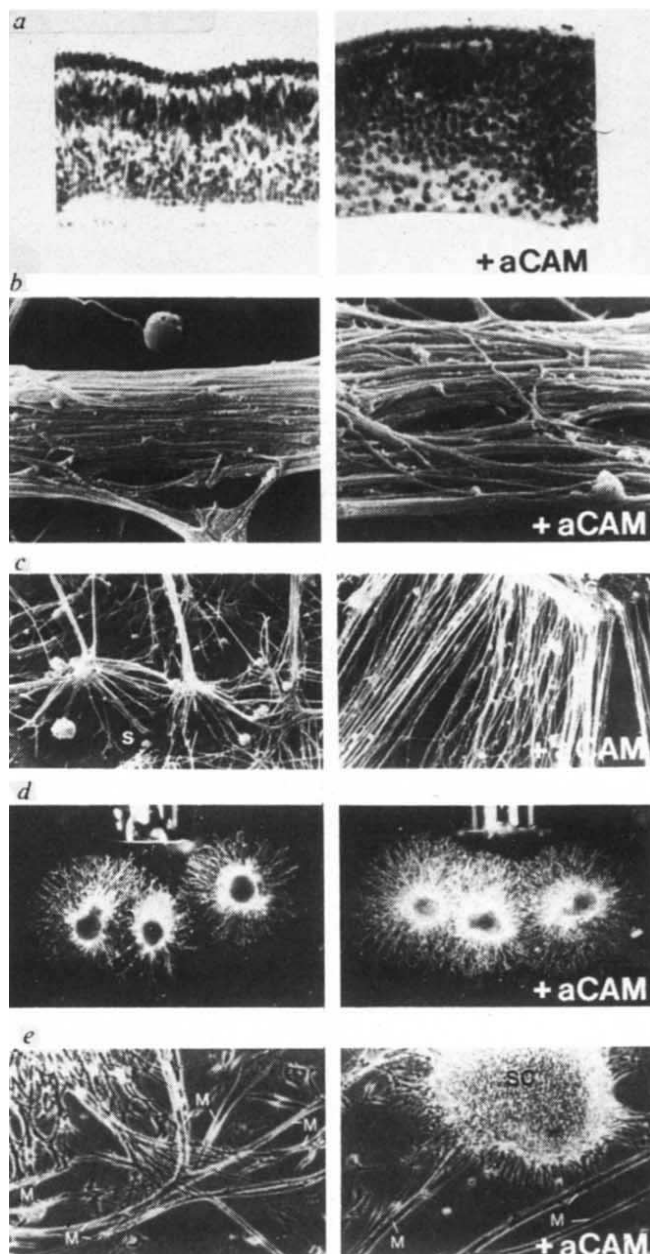


Fig. 3 *In vitro* studies of N-CAM function in neural development. **a**, Effect of anti-N-CAM Fab (denoted aCAM on the figure) on differentiation of cultured retinal cell tissue⁴¹. Note that the formation of discrete cell and neurite layers (left) is prevented by the antibody (right). **b**, Requirement for N-CAM-mediated binding in the formation of stable neurite fascicles. The presence of anti-N-CAM Fab causes nerve bundles to split apart and to exchange neurites with other fascicles⁴². **c**, Alteration of fascicle branching patterns by anti-N-CAM Fab. Branching reflects a competition between adhesion among neurite shafts and the pulling force exerted by growth cones as they migrate along the substrate⁴². When Fab is added, the growth cones dominate and the fascicles are completely dispersed. **d**, Growth of dorsal root ganglion neurites towards a capillary source of NGF⁴³. Only the initial neurite outgrowth is actively attracted to the capillary (see text), and the marked asymmetry shown on the left requires growth of subsequent neurites along their predecessors. In the presence of anti-N-CAM Fab, this 'tracking' mechanism is inhibited and a more symmetrical pattern of outgrowth is obtained (right). **e**, N-CAM-mediated adhesion in initial interactions between spinal cord neurones and myotubes (M) in culture⁴⁵. Left: control culture with neurite outgrowth extending to, traversing and interconnecting the myotubes. Right: culture with anti-N-CAM Fab in which nerve-muscle interactions are too unstable to support extensive neurite outgrowth from the spinal cord explant (SC).

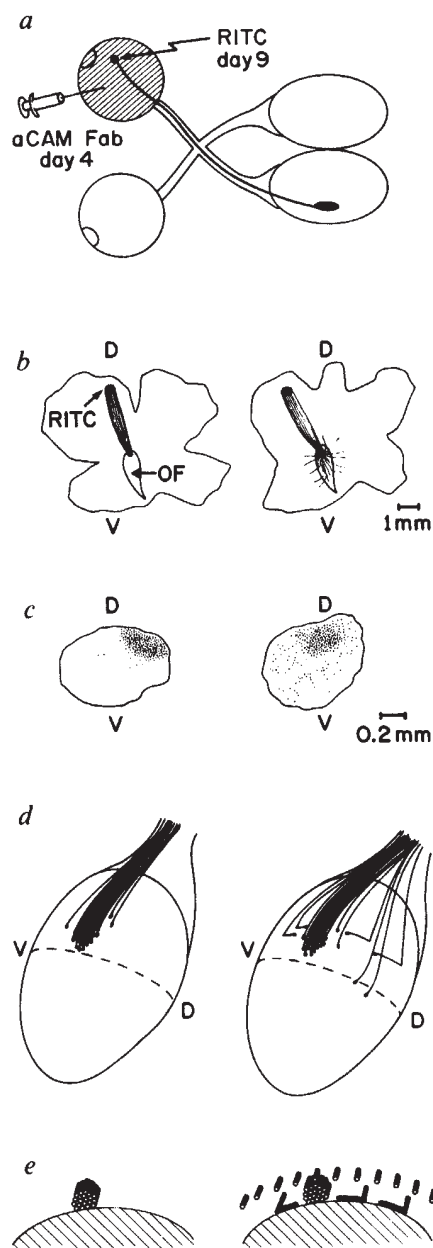


Fig. 4 Effect of intraocular injection of anti-N-CAM Fab on development of the chick retinotectal projection⁴⁷. *a*, Diagram of experiment using Fab injection on embryonic day 4 to perturb adhesion, and localized uptake of rhodamine isothiocyanate (RITC) on day 9 to label ganglion cell axons originating from a distinct region of the retina. The antibody remains within the eye and persists for at least 2 days. *b*, Intra-retinal routes of RITC-labelled fibres. In normal development (left), the labelled fibres remain together and occupy a distinct region in the optic fissure (OF). With the Fab injection (right), the initial path of the axons is normal, but many fibres become misrouted at the fissure and leave the eye at ectopic positions. Some fibres even fail to exit at the fissure. *c*, Cross-sections through the optic nerve of a control embryo (left) in which the RITC-labelled fibres remain localized in the appropriate part of the bundle, and of an Fab-injected embryo (right) where, in the absence of antibody, the misrouted fibres from the fissure remain in dispersed, ectopic positions. *d*, Routes of RITC-labelled fibres on the tectum in a control (left) and Fab-treated (right) embryo. Without antibody perturbation, fibres originating from dorsal retina grow parallel to each other and terminate in the appropriate site on ventral tectum. The ectopic fibres of Fab-injected embryos either make right-angle corrections in their dorsal-ventral position or grow in straight paths. As shown diagrammatically in *e*, the RITC-labelled fibres capable of correcting their position grow in closer contact with the intermediate zone of the tectum (hatched area), whereas the non-correcting population represents fibres which grow on top of other, unlabelled fibres, and are therefore less intimately associated with cell bodies of the intermediate zone.

With stronger growth cone-substrate interaction, the nerve bundles tend to be pulled apart at their ends and in the presence of anti-N-CAM Fab the pattern of outgrowth is dominated by the dispersed distribution of the neurite tips (Fig. 3c). These observations suggest that the branching pattern of a nerve bundle reflects a competition between N-CAM-mediated adhesion among neurite shafts and the defasciculation caused by the pull of growth cones as they migrate along the substrate⁴².

Tracking of growth cones along nerves. Fascicles can be produced either by side-to-side adhesion among neurite shafts or by growth of one fibre along another. The second mechanism is common *in vivo* and can be a dominant factor in the formation of major nerve pathways. A model for nerve 'tracking' has been studied⁴³ in which a capillary tube filled with nerve growth factor (NGF) is used to produce a transient gradient for attraction of neurites (Fig. 3d). The NGF gradient disappears before most of the neurite outgrowth occurs, so that the observed asymmetry primarily results from growth of the later fibres along the pathways taken by the earlier fibres. Inhibition of adhesion by anti-N-CAM Fab prevents this tracking, so that most of the neurite outgrowth distributes uniformly around the ganglion.

Nerve-muscle interaction. Muscle cells express N-CAM on their surface, and nerve membranes can adhere to them via this molecule⁴⁴. In cultures of spinal cord explants with skeletal myotubes, nerve fibres slowly migrate on the substrate until they make contact with a muscle cell. Once such a bridge is established, it serves as a track for rapid growth of many nerves to the myotube⁴⁵. The growth cones then migrate along the myotube, causing the nerve bundle to split apart (Fig. 3e). When anti-N-CAM is present, the nerve fibres are unable to use the muscle cells as a substrate and fail to produce fascicles of substantial length. When a more adhesive tissue culture dish is used, the neurites continue to elongate, but pass the myotubes without changing direction. It is unlikely that synapses can form in such conditions, and together, these observations suggest that N-CAM-mediated adhesion is an obligatory early step in nerve-skeletal muscle recognition⁴⁵.

Effect on visual system development

The remainder of this review will focus on one aspect of neural development in order to integrate much of what is known about N-CAM, and because the visual system has provided the most extensive studies *in vivo*. The two key experiments in this analysis have been the characterization of N-CAM carbohydrate in different parts of the visual system⁴⁶ and the injection of Fab into the eye-cup of a 4-day chick embryo followed by tracing of retinal ganglion cell axon pathways (Fig. 4a)⁴⁷.

At embryonic day 10, the N-CAM isolated from chick neural retina has less sialic acid than has N-CAM from the rest of the central nervous system¹⁸. Because of the potential influence of such differences on cell-cell interaction, the forms of N-CAM in the visual system were analysed in greater anatomical detail⁴⁶. These studies indicated that N-CAM from both neurite and nuclear layers of the neural retina of 5–10-day old embryos exists in a similar, relatively sialic acid-poor form. In contrast, the optic nerve beyond the eye, as well as the optic tract and tectum, each contain sialic acid-rich N-CAM. Retinal ganglion cells are particularly interesting in that their perikarya and intraocular neurite shafts apparently have N-CAM of a lower sialic acid content than the portion of their axons that extends beyond the fissure to the tectum. The possible effects of this regional difference are discussed below in the context of the Fab-injection experiment and development of the retinotectal projection.

Retinal ganglion cells begin to send their axons to the tectum during the third day of chick embryogenesis⁴⁸. The first step in formation of the optic nerve is the growth of axons in straight radial paths along the inside of the retina. In this region of the eye, intraocular injection of anti-N-CAM Fab did not seem to disrupt fibre order or direction (Fig. 4b), possibly because the fascicles are guided and supported by channels constructed from the endfeet of glial cells⁴⁹. In the chick, this channel system

ends abruptly at the fissure, where all fibres must coalesce in an orderly manner to form the optic nerve⁴⁹. The fissure is exactly the site at which the antibody causes major deviations in the routes taken by ganglion cell axons (Fig. 4b), resulting in the formation of an optic nerve in which local retinotopic order has not been maintained (Fig. 4c).

This effect of the Fab suggests that the initial orderly assembly of an optic nerve requires particularly stable fasciculation. The lower sialic acid content of intra-retinal N-CAM may well reflect this requirement, in that it would provide greater adhesivity among retinal ganglion cell axons. Beyond the eye, the configuration of fibres within the optic nerve undergoes a variety of alterations including inversion, interdigitation with the contralateral nerve at the chiasm and a progressive spreading as the nerve approaches the tectum and the axons splay out over the tectum to synapse with appropriate targets⁴⁵⁻⁵³. While the preservation of local fibre order throughout the pathway⁵³⁻⁵⁷ suggests that a sufficient adhesion occurs among the axons to maintain fascicle stability, there must also be sufficient plasticity to allow these alterations in the overall structure of the optic nerve. In this respect, it is striking that the N-CAM isolated from the optic nerve and tectal membranes has a high sialic acid content, which could provide the less avid adhesion required for these events. Once the projection has been established, such plasticity would presumably no longer be required. It can be argued, therefore, that the gradual loss of sialic acid from optic nerve and brain N-CAM during the final stages of maturation^{21,46,58} might in part serve to fix the retinotectal pathways established during embryogenesis^{3,46}.

Retinal ganglion cell axons grow out of the chick eye over a period of 2-3 weeks, arising first from cells in the central-dorsal region of the retina and then from an expanding ring of cells surrounding this site⁵². During this time the optic nerve gradually enlarges by growth of new axons between an outer layer of neuroepithelial cell endfeet and the axons of their predecessors⁴⁸. This process continues throughout the pathway, so that at the tectum the later-arriving axons are separated from the intermediate zone of the tectum by an increasing fibre layer^{52,53}. In the Fab-injection experiment, retinal ganglion cell axons are no longer exposed to the antibody after leaving the eye, and the fate of the misrouted fibres during optic nerve development could be examined in otherwise normal developmental conditions. These experiments have led to three observations: (1) disorder produced in the fissure persists throughout the optic pathway to the tectum (Fig. 4c, d), (2) many of the misrouted fibres which are closer to the intermediate zone of the tectum make dramatic corrections in their position (Fig. 4d, e) and (3) ectopic axons which are growing on top of the fibre layer grow in straight tracks on the tectum and therefore do not correct their position (Fig. 4e). These observations suggest that early-arriving fibres at the tectum can read some type of positional marker⁵⁹⁻⁶³ along the dorsal-ventral axis. However, as development proceeds, these markers become obscured by

the increasing fibre layer, and it becomes necessary for the late-arriving axons to use a different type of guidance. This seems to be a tracking mechanism, analogous to that observed in the NGF capillary experiment described above, which in effect preserves the tectal guidance cues and would also help to maintain local order among the axons. Thus, the role of N-CAM adhesion in development of the retinotectal projection seems, rather than selective cell-cell recognition, to be more a supportive function whose regulation allows the expression or maintenance of other sources of specificity.

Concluding remarks

There is now sufficient information about the chemistry and biology of N-CAM-mediated adhesion to begin to interpret its role in the development of intact embryos, especially the nervous system. However, many assumptions are still included in these analyses, and it will be particularly important to obtain a more complete account of N-CAM structure as it relates to the formation of cell-cell bonds. With respect to embryogenesis, many intriguing questions remain. Is the function of N-CAM adhesion similar at all stages of development? Perhaps the early embryo uses it simply to produce multicellular assemblies whereas later roles include the final structuring of differentiated tissue. At the molecular level, is the heterogeneity in sialic acid content caused by factors intrinsic to the cell or does it result from modifications imposed by the environment? Also, because N-CAM is probably not the only cell-adhesion ligand even in neural tissue, it will be important to determine whether combinations of two cell adhesion mechanisms could³⁶ result in cell-cell interactions distinct from those produced by either alone.

In summary, it might be most appropriate to consider N-CAM as a versatile glue that serves to hold cells together during a variety of developmental events. The molecule's function seems to be regulated by spatiotemporal differences in both its expression and structure, which may provide for a certain degree of binding specificity among cells. Recent evidence suggests, in fact, that guidance of optic axons from the retina to the tectum involves adhesion of their growth cones to a preformed adhesive pathway produced by preferential expression of N-CAM on endfeet of certain neuroepithelial cells⁶⁴. On the other hand, many of N-CAM's contributions to tissue formation involve the effects of adhesion on other developmental parameters, rather than a direct influence on cell or axon position according to surface affinities. In this case, regulation of the molecule's function could provide for specific membrane contacts during critical phases of cell-cell communication, and produce permissive or non-permissive conditions for remodelling (and possibly regeneration) of tissues and neuronal connections.

I thank Jerry Silver, Raymond Lasek, Michael Katz and Norman Robbins for many helpful comments, and Mary Ellen Bleakley for manuscript preparation. The work represented here was supported in part by USPHS grants HD-09635 and HD-18369 from the NIH.

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ARTICLES

Broad-line high-excitation gas in the elliptical galaxy NGC5128

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A faint, but extensive component of broad-line ionized gas has been discovered in the peculiar giant elliptical galaxy NGC5128. This component has a radically different spatial distribution from the well-studied rotating photoionized gas associated with the dust lane although the velocity fields of the two components are similar. The origin of the broad-line gas is considered and its possible relation to the active nucleus and the X-ray jet discussed.

THE extensive emission-line regions associated with the dust lane of the peculiar giant elliptical galaxy NGC5128 (Centaurus A) were first observed over 60 yr ago by Hubble¹. This gas, which consists of both discrete H II regions centred on clumps of blue stars and a more diffuse component covering the dust lane, is photoionized by the radiation of the many young, hot stars found in the same general area^{2–5}. Both the gas and the hot stars lie in a disk or ring structure which rotates in a more or less uniform and organized fashion^{2,6–8}. It is now widely accepted that this gas disk is the product of a recent merger^{9–11}.

The filaments discovered by Blanco *et al.* constitute the second component of ionized gas in NGC5128¹². These are roughly aligned between the nucleus and the outer north-east radio lobe. Although clumps of blue stars are found in association with the filaments, the dominant source of excitation of this gas is probably nonstellar^{13,14}. That the filaments are in some way connected with the north-east radio lobe seems certain^{14,15}.

Evidence^{4,5} for yet a third distinct component (component III) of ionized gas in NGC5128 has been found in the vicinity of the nucleus and the so-called 'knot X' region² on the south-west edge of the dust lane. The component III gas is characterized by general turbulence and relative emission-line intensities which, as in the north-east filaments, are inconsistent with normal photoionization by starlight. The lack of information on the spatial distribution and kinematics of component III has, however, made it difficult to decide on its true nature.

We now present interferometric observations of NGC5128 designed specifically to address these questions. Our results unequivocally demonstrate that the component III gas found

at the nucleus and knot X is part of a much larger diffuse region or broad emission which is extended along an axis approximately orthogonal to the plane of the dust lane, and which, therefore, may be associated with the main body of the giant elliptical. The observed radial velocities show a gradient similar to that of the well-studied rotating dust lane component (component I), but with considerable small-scale structure. We consider the origin of component III in the light of these new data.

Interferometric observations

NGC5128 was observed for 220 min in the [O III] λ 5,007 line, through an interference filter 20 Å FWHM, on the night of 11 March 1981 using the Taurus imaging Fabry–Perot system^{16–18} at the Cassegrain focus of the Anglo-Australian Telescope (AAT) 3.9-m telescope. The detector used was the AAT Image Photon Counting System (IPCS)¹⁹. An area approximately 4.9×4.9 arc min² centred roughly on the nucleus was covered with a pixel size corresponding to 1.85×1.85 arc s² on the sky.

Low-resolution spectra show that the [O III] λ 5,007 line is one of the strongest emitted by component III, whereas its relative intensity is quite weak in the low-ionization dust lane component I^{4,5}. Hence [O III] λ 5,007 offers much better contrast between components I and III than H α , the more traditional line used in velocity field observations. Our measurements consisted of 120 spectral increments (each 160×160 spatial pixels) covering $1,022 \text{ km s}^{-1}$, with a free-spectral range of 930 km s^{-1} and giving a spectral resolution of 26 km s^{-1} FWHM. Standard techniques were used to calibrate the data^{18,20}.

Results

The fully-calibrated three-dimensional Taurus data cube was initially smoothed by a three-dimensional gaussian having a spectral FWHM of 15 km s^{-1} and a spatial width of 2.3 pixels

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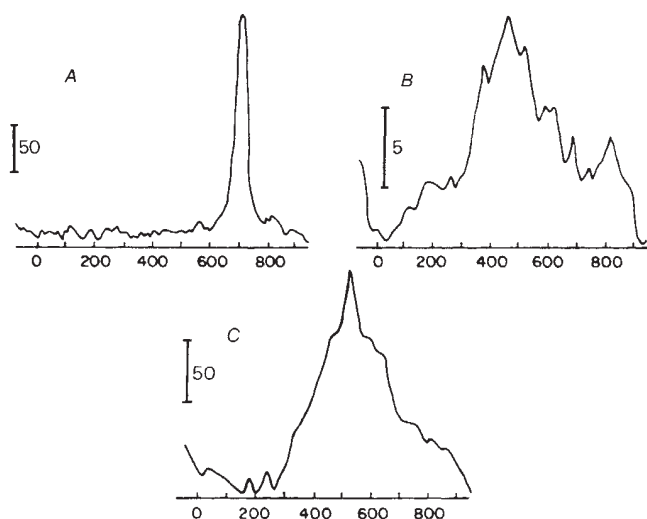


Fig. 1 Sample [O III] $\lambda 5,007$ line profiles observed with Taurus at the positions indicated in Fig. 2. Note the wrap-around of the spectra due to the 930 km s^{-1} free-spectral range. The intensity scale, in terms of accumulated counts, is given alongside the vertical bar for each profile. By comparison, the background stellar component gives an approximately sinusoidal 6% modulation with a period corresponding to this free-spectral range, and hence the broad line structure seen in the displayed profiles is real.

(4.3 arc s). Inspection of these data revealed two quite separate components to the [O III] emission in NGC5128. The first, a narrow-line component with a FWHM averaging 55 km s^{-1} and a spatial structure similar to that of the $\text{H}\alpha$ distribution in the disk of NGC5128 (refs 7, 8), was clearly associated with the [O III] emission from the H II regions in the disk (component I). However, superimposed on this was a much broader emission-line region whose surface brightness was, in general, significantly lower than of the narrow-line H II region gas. This broad component was unquestionably an emission line as the underlying stellar continuum only showed an $\sim 6\%$ modulation with a period corresponding to the free spectral range. Comparison with the work by Mollenhoff⁴ and Phillips⁵ clearly identifies this gas as component III.

Samples of [O III] line profiles from the Taurus data are shown in Fig. 1. (Positions are indicated in Fig. 2). Profiles A and B are representative of the narrow and broad components, respectively. Profile C is an example of a region close to knot X where both narrow and broad structure is observed. Here the broad-line component is very strong and, although non-gaussian, has a FWHM of 350 km s^{-1} , whereas the narrow-line component has a FWHM of only 40 km s^{-1} .

To examine both the spatial distributions and velocity fields of the broad- and narrow-line regions separately, we first used an algorithm which obtained for each spatial pixel, an emission-line flux estimate for the narrow component alone. The spatial distribution of this narrow-line flux map is exactly that expected for the H II regions in the disk of NGC5128, as shown in Fig. 2a. Using this narrow-line region flux map as a logical mask, gaussians were fitted to all pixels within these regions and a velocity field obtained.

In the vicinity of knot X and the nucleus of NGC5128, the broad-line emission has a surface brightness comparable with that of the narrow-line gas. However, there was clear evidence that this broad-line component extended well beyond the disk into the elliptical galaxy component to the north-east and south-west of the nucleus at a very much lower surface brightness. To examine the spatial structure and velocity field of this weaker broad-line emission, it was necessary to smooth the three-dimensional data by a two-dimensional spatial gaussian of FWHM of 20 pixels (37 arc s). Spectral gaussians were again

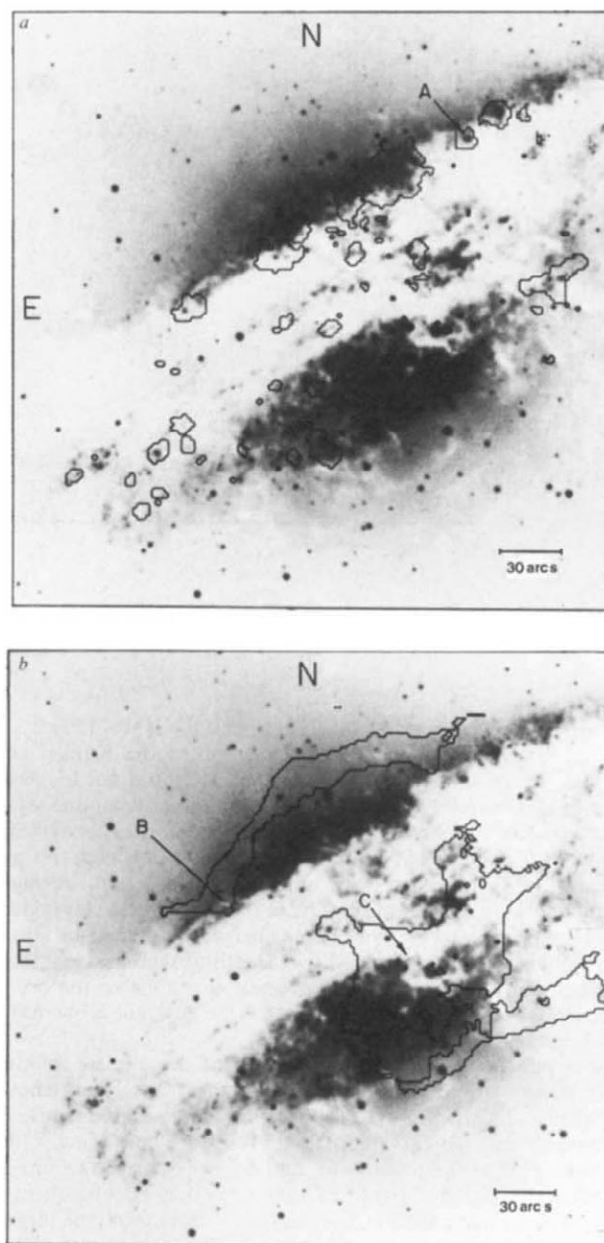


Fig. 2 a, Distribution of narrow ($\text{FWHM} < 150 \text{ km s}^{-1}$) [O III] $\lambda 5,007$ emission in NGC5128 superimposed on a blue-violet photograph. This emission is identified with the well-studied dust lane component I. b, Distribution of broad ($\text{FWHM} \geq 150 \text{ km s}^{-1}$) [O III] $\lambda 5,007$ emission.

fitted to all pixels which showed appreciable signal in the broad-line component. Clearly, this heavy smoothing mixed in the narrow-line regions with the more diffuse broad-line gas over length scales of approximately 20 pixels, and thus we excluded all fits which gave lines whose FWHM was $< 150 \text{ km s}^{-1}$, which is about three times the average width of the narrow-line H II region gas.

The resulting broad-line gas distribution is shown in Fig. 2b. Obviously, the detectability of the broad-line component is influenced by the strength of the narrow-line emission in the disk. For example, the slit spectra published by Graham² would indicate that the emission from the strong narrow-line gas along the north-east edge of the dust lane and that which arises in the bordering 'rim' of broad-line gas associated with the elliptical galaxy component merge smoothly instead of there being a gap as suggested by Fig. 2. Furthermore, as indicated in Fig. 6 of

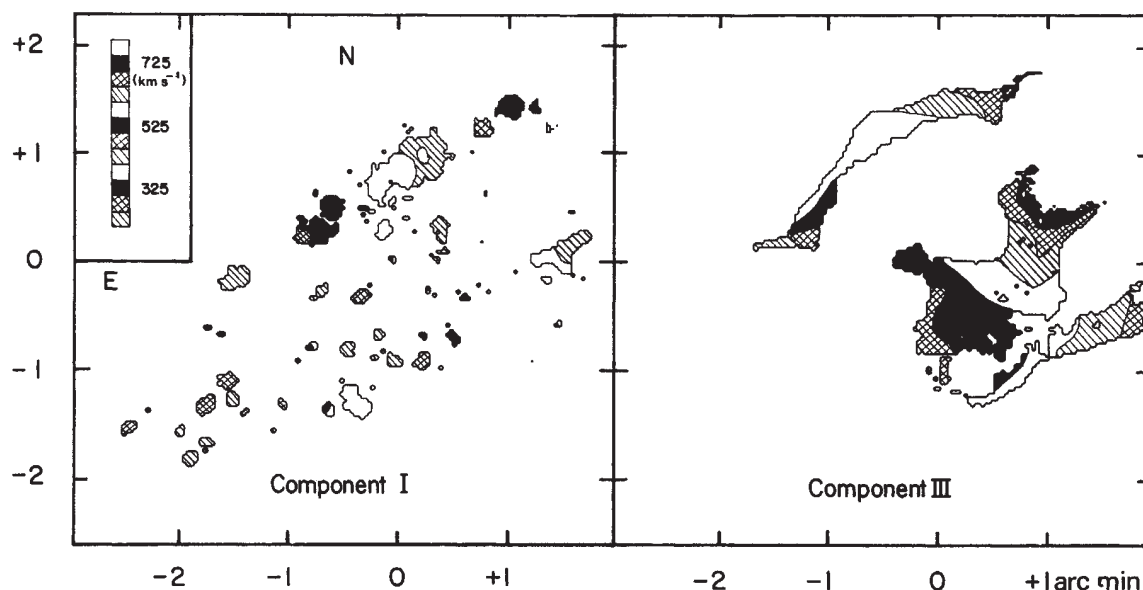


Fig. 3 Two-dimensional representation of the radial velocity fields of components I and III. The largest velocity measurements occur in the north-west and the smallest in the south-east.

ref. 5, some of the component III gas has a FWHM $<150 \text{ km s}^{-1}$ (a case in point being the exact location of the Kunkel and Bradt²¹ near-IR nucleus). Hence, Fig. 2b should not be taken as an exact representation of the distribution of component III. Nevertheless, the broad-line gas clearly has little, if anything, to do with the dust lane and disk of hot stars. Instead, this gas seems to be associated with both the nucleus and the main elliptical body of the galaxy. (Note that this broad component is observed to the north-west of the nucleus in the same locations where near-IR photographs show the elliptical component shining through the dust lane. Conversely, no trace of the broad emission is seen to the south-east where the dust lane is obviously thicker.)

Comparison of the radial velocities of the narrow rotating component I as derived from the Taurus [O III] data shows generally excellent agreement with the measurements of Graham², and Taylor and Atherton⁸. More surprisingly, the velocity field of the broad-line component III, as shown in Fig. 3, generally displays a gradient similar to that of component I, but with several noteworthy small-scale deviations, the largest of these having previously been noted by Graham² at the position of knot X. Another exceptional region is located $\sim 2 \text{ arc min}$ to the south-west of the nucleus. Here, double emission is observed over a wide area, with the radial velocity of the broad component averaging more than 100 km s^{-1} greater than that of the narrow component. These deviations, together with the broad line widths, reinforce the conclusion that component III is characterized by considerable turbulent motion⁶. The key question, which has to be addressed if we are to understand the nature of this gas, is how do we reconcile the broad line widths with a velocity field which is similar to that of component I?

Conclusions

The present observations reveal that the peculiar emission-line gas of component III is very extended, and show that it has a distinctly different morphology from that of the disk of ionized gas associated with the dust lane (component I). The distribution of component III correlates well with the main elliptical body, but show a velocity gradient similar to that of the disk. The latter would seem to imply either that both components I and III share a common origin, or that considerable interaction between the two has occurred. Clearly any model which seeks to explain the origin of the gas in NGC5128 must also account for the differing properties of components I and III.

Several explanations for these observed properties of component III come to mind. X-ray imaging observations of NGC5128 suggest the existence of an extensive component of hot gas trapped in the potential well of the main elliptical galaxy body²². As has been suggested for several other nearby radio ellipticals (such as M87 (ref. 23), NGC127 (ref. 24) and NGC4696 (ref. 25), component III might be cooling condensations in the flow of this much hotter gas onto the nucleus^{26,27}. However, Graham² has shown that the rotation of the elliptical component is very small. Therefore, the zero-intensity full width of $\approx 900 \text{ km s}^{-1}$ that we observe for component III is clearly much larger than expected from the line-of-sight integral through the elliptical component.

Alternatively, components I and III might be the inner and outer portions, respectively, of a disk which is warped in the plane along the line-of-sight (that is perpendicular to the line of nodes)⁸. In this scenario, it is possible that the gas in component III is directly photoionized by high-energy photons emitted by the active nucleus. The chief attraction of this explanation is that it accounts for the similar kinematics of components I and III, but it is again hard to envisage a method or producing the observed line widths.

We consider it more likely that component III is a product of the unusual nuclear activity of the galaxy. It is striking that knot X, the nucleus, and the X-ray^{22,28} and radio²⁹ jets in NGC5128 are almost exactly colinear. Furthermore, the extensive area of component III emission south-west of the nucleus (see Fig. 2b) lies nearly precisely on the peak of the radio emission of the inner south-west radio lobe²⁹. This circumstantial evidence suggests that component III shares the bipolar geometry of the inner radio lobes and lies more or less perpendicular to the plane of the disk component I. Graham² has argued that the north-east edge of the disk component is nearest to us, which would explain why the component III gas to the south-west can be traced into the nucleus, whereas it is observed to the north-east only beyond the disk rim. In this interpretation, component III might be gas ejected in an approximately biconical pattern from the nucleus, or cooling condensations in the ambient interstellar medium of the elliptical galaxy body which has been compressed and heated through interaction with the inner radio lobes. The major difficulty with either of these hypotheses is explaining why the gradient in the velocity field of component III is so similar to that of the disk component I. If ejection is the correct model, perhaps we are witnessing the

impact of the expelled material with the disk gas. This would be consistent with the considerable turbulent velocity structure observed in component III.

Although there is a strong possibility of a link between the nuclear activity in NGC5128 and the existence of component II, a significant conclusion of this work is that there is no evidence in the Taurus [O III] images for a line-emission counterpart to the actual X-ray and radio jets. In particular, the three regions of line emission recently observed by Brodie *et al.*³⁰, which these authors claimed to be associated with knots in the inner 2 kpc of the X-ray and radio jets, would appear instead to be unrelated samples of the much larger distributions of ionized gas which comprise components I and III.

At least two follow-up observations are suggested by our results. First, the ionization mechanism of component III should

be investigated in more detail, as the low-resolution spectroscopic data obtained to date are confined to the relatively bright knot X and nucleus regions, with the ionization characteristics of much of the fainter regions of component III remaining largely unknown. Second, high-dispersion spectra with better spatial resolution and at a higher signal-to-noise ratio should be obtained to determine accurately the profiles of the broad-line component III gas. Such observations would test the conical ejection hypothesis, as this model predicts oppositely-skewed line profiles to the north-east and south-west of the nucleus.

Judging from the new data we have presented, there is still much to be discovered of the complex phenomenon occurring in this, our nearest example of a giant elliptical radio galaxy.

We thank the staff of the AAT for technical support, and Nelson Caldwell, Jay Frogel and John Graham for comments.

Received 10 January; accepted 19 June 1984.

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Large, highly organized radio structures near the galactic centre

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A radio map of the galactic centre made at a wavelength of 20 cm with the Very Large Array telescope reveals that most of the radio emission arising within 50 pc of the galactic centre is not associated with ionizing radiation from recently formed stars. Rather, the large-scale geometry and the measured polarization of the radio emission strongly suggest that the nonthermally emitting gas is arranged along magnetic structures indicative of a substantial poloidal component to the magnetic field in the central region of the Galaxy.

THE centre of our Galaxy has been the focus of much attention (see refs 1, 2) because of its potential significance for our understanding of the source of activity in galactic nuclei. Much work has dwelt on the small-scale radio and IR structure of Sgr A, the apparent centre of luminosity and dynamical centre of our Galaxy^{3–12}. On a larger scale (10–100 pc), several low-resolution studies have been carried out, revealing the distribution of molecular clouds, ionized gas and warm dust^{13–23}. Such studies are beginning to shed light on the spatial relationships between the ionized, atomic and molecular components of the interstellar environment near the galactic nucleus. However, many fundamental problems remain: for example, what is responsible for the ionization and what is the relationship between the dust and the ionized gas? Population I stars have been considered as the source of ionizing photons²⁴, but direct evidence for the presence of an adequate number of OB stars is lacking.

We have used the Very Large Array (VLA) telescope of the National Radio Astronomy Observatory (NRAO) to obtain a high-resolution view of the radio continuum emission within a

relatively large field of view (25 arc min at 20 cm wavelength, or about 75 pc). The field of view at 20 cm was offset from the galactic centre in order to include not only Sgr A and its radio halo, but also the entirety of the prominent radio structure lying to the north-east of the galactic centre: the 'continuum arc' (hereinafter referred to as the 'Arc'). Smaller fields observed at 6 cm wavelength were positioned along the ridge of this Arc. The choice of these directions was motivated in part by the interferometric results of Downes *et al.*²⁵, who reported several compact sources coincident with the Arc. Our observations reveal that the Arc consists of a highly ordered set of large-scale features which are probably related to a galactic-scale phenomenon rather than to localized star formation in pockets near the galactic centre. Therefore, the case for newly-formed OB stars as the primary source of ionization is weakened.

The Arc was evident in a map made as early as 1959^{26,27}. Subsequent radio maps of the galactic centre have shown the Arc more or less clearly at frequencies from 0.4 to 15.5 GHz (refs 17, 28–33). The 10-GHz map of Pauls *et al.*¹⁷ made with the 77 arc s beam of the 100-m telescope at Bonn presents the

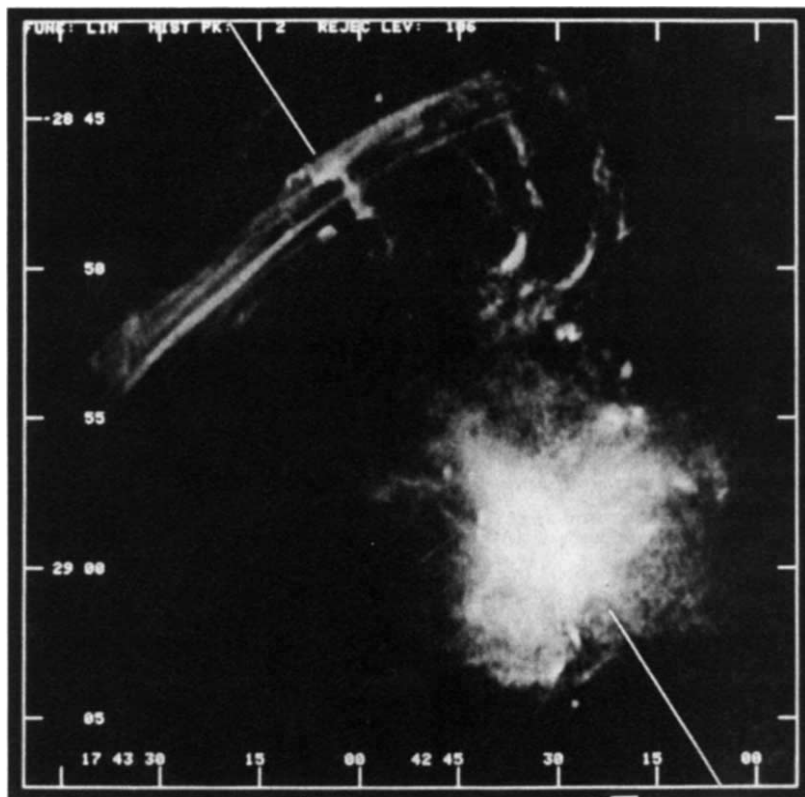


Fig. 1 Radiograph of large-scale, 20-cm radio emission features within ~ 60 pc (20 arc min) of the galactic nucleus. The line shown is parallel to the galactic plane at galactic latitude $b = -0.05^\circ$. The contrast is chosen to bring out radio features brighter than 15 mJy per beam area (the half-power beam width is 5×9 arc s). This map has been corrected for the primary beam response of individual antennas, which has a full width at half power of 28 arc min. The galactic nucleus lies at $\sim \alpha(1950) = 17 \text{ h } 42 \text{ min } 30 \text{ s}$, $\delta(1950) = -28^\circ 59'$.

most detailed picture obtained until now. The Arc appears to consist of a continuous, narrow strip of radio emission which is perpendicular to the galactic plane at about $l = 0.18^\circ$, and which is sharply bent at $l \sim 0.16^\circ$, $b \sim 0.06^\circ$, so that it appears to merge with the diffuse halo of Sgr A from the north.

Observations

The observations were carried out at both 6 and 20 cm, with the VLA in two different configurations. In September 1982, we used the relatively large B array, in which the telescope spacings ranged up to 11 km. The configuration used in 1983 (the hybrid C-D array) was essentially an order of magnitude more compact.

The data from the two array configurations were combined to produce a map based on sampling over a large range of spatial frequencies. The procedure used to combine and self-calibrate the data, and then to deconvolve the map from the synthesized beam (the CLEANing algorithm) will be described elsewhere. The spatial resolution in the 20-cm map was $\sim 4.4 \times 9$ arc s (RA $\times$ Dec). A dynamic range of ~ 300 was obtained in the final, 20-cm CLEANed map. Such a large dynamic range is necessary to bring out the details of the Arc structure. Still, this map is dynamic-range limited, with an r.m.s. noise of ~ 5 mJy per beam area. The 6-cm maps could not be self-calibrated because of the absence of adequately strong point sources, so their dynamic ranges are smaller, ~ 40 . The r.m.s. noise in the 6-cm maps reported here (using C-D array data only) is 1–4 mJy per beam area. At 20 cm, all of the radio structure relevant to the present investigation is well localized within the primary beam of a single antenna, so that the corrections for the primary beam responses are not large enough to introduce any great uncertainties. At 6 cm, on the other hand, this correction can be quite substantial for emission arising near the edges of the fields.

Sgr A and its halo are at the core of a very extended distribution of radio emission^{24,33}. Because we have not attempted to account for flux present at scales larger than that which corresponds to the spatial frequency of the shortest antenna baseline, our maps do not accurately represent the extended flux. At 20 cm (6 cm), features which are extended over more than ~ 11 (4) arc

min are under-represented or missing. Our conclusions are not affected by this limitation, however.

Results

The most interesting new result from our observation is the recognition that the Arc consists of a system of narrow filamentary structures having typical widths of ~ 1 pc and lengths > 30 pc. These are best seen in the map made at 20 cm wavelength of the entire region surrounding Sgr A and the Arc (Fig. 1). The radio filaments can be grouped into two categories on the basis of their geometry and apparent physical characteristics: (1) the vertical filaments: those located at $l = 0.16\text{--}0.18^\circ$ which are perpendicular to the galactic plane, parallel to each other, and strikingly regular, unbroken and homogeneous in their appearance, and (2) the 'arched' filaments: those that arise (or end) in the confused, northern part of the low surface brightness halo of Sgr A and diverge, curving eastward, until they join the filaments in the first category. The 6-cm map of the arched filaments, made with the data from the C-D hybrid array alone, is shown in Fig. 2.

Remarkably, these two systems appear to connect at roughly a right angle. One is tempted to call this a real physical connection, as neither set of filaments overshoots the region where the connection seems to take place. However, the correspondence between filaments in the two systems is unclear because the emission is relatively weak in this area.

The 'vertical' and the 'arched' filaments differ in a few important respects. The arched filaments have a far-IR counterpart suggesting that these may be primarily thermal features, while the vertical filaments have no such counterpart^{20,22,34}, indicating that their radio emission may be predominantly non-thermal. These characteristics are consistent with, but not proven by, the spectral index information. Second, the vertical filaments show linear polarization at a few places along their length, while the arched filaments have no significant polarization. Again, this is consistent with their suggested thermal and non-thermal natures, respectively. Another difference is that the brightness temperature of the vertical filaments is quite uniform—typically 1,000 K along their ridges—except for a brightening where they

join the radio feature G0.18–0.04 (that is, where they cross the galactic plane). The arched filaments vary more in intensity and are generally dimmer.

The vertical filamentary segments have a slight curvature, convex towards increasing longitude. (This is evident in earlier single-dish maps, see ref. 17.) The centre of curvature lies in or near the galactic plane, and cannot be identified with the galactic centre unless the true curvature is substantially greater but is diminished to our view because of a large projection angle. In that case the actual distance separating the filaments and the galactic centre would be ~ 6 times the apparent distance.

The relatively bright vertical filaments have a projected length of ~ 40 pc. They end rather abruptly, especially at the southern end (Fig. 1). However, the 6-cm map made by Altenhoff *et al.*³³ indicates that the broader envelope of this system of narrow, linear structures is continuous (at a low surface brightness, but clearly in excess of the background) out to almost a full degree (180 pc) in both directions away from the galactic plane. This is an order of magnitude greater than the length of the relatively bright vertical filaments in Fig. 1. As there is no hint of this extension in our data (at either 6 or 20 cm) it must be relatively smooth and broad. Interestingly, these extensions appear to preserve the curvature seen in the vertical filaments by the VLA.

One of the intriguing characteristics of the vertical radio filaments is the apparent doubling of at least two of them; a dark line appears to split the two brightest filaments. These may indicate the superpositions of two separate filaments in each case, or, alternatively, the dark lines may indicate that the filaments consist of two (or more) strands.

On a much larger scale, helical structures appear to be present: the low-brightness emission surrounding the Arc appears to consist in part of three helical segments whose axis coincides with the mean position of the filaments. The apparent helical segments are brightest near $\alpha = 17^{\text{h}} 43^{\text{m}} 25^{\text{s}}$, $\delta = -28^{\circ} 48'$ (see Fig. 1). They add a third dimension to the appearance of the Arc. At the positions where the large-scale helical features and the vertical filaments are superimposed, it is clear that the helical structures are passing in front of the vertical filaments; the brightness of the vertical filaments is clearly diminished along a line coinciding with an extrapolation of the helical segments across the filaments, as would be expected if the helical features were optically thick in the continuum. If the helical features are optically thick, however, then they are cool, because their brightness temperature is only ~ 500 K.

Not all of the radio emission from the Arc arises in filamentary structures (or their presumably associated helical features). At about the position where the vertical filaments cross the true galactic plane, a radio structure (referred to in single-dish studies as G0.18–0.04) is seen superimposed on the filaments. As can be seen in the 6-cm C–D map of this object (Fig. 3), it is crudely sickle-shaped, oriented transverse to the long axis of the filamentary system. The maps strongly suggest an interaction between the filamentary structures and G0.18–0.04: first, the northernmost filament brightness substantially as it nears the semicircular portion of G0.18–0.04. Also, G0.18–0.04 appears to loop over, and then roughly merge with the northernmost filament. Although not evident in the figures presented here, the southernmost bright filament appears to cast a shadow where it crosses the 'sickle' structure. Finally, a filament appears to begin or end abruptly on the small but resolved double source at $\alpha = 17^{\text{h}} 43^{\text{m}} 05^{\text{s}}$, $\delta = -28^{\circ} 48' 42''$, which is apparently part of G0.18–0.04.

VLA observations of the bright emission from Sgr A (the radio complex having a diameter of ~ 10 pc and incorporating the galactic nucleus) were recently reported by Ekers *et al.*¹⁰. Because we have used here the most compact configuration of the VLA and have achieved a relatively large dynamic range, we are sensitive to weaker, larger-scale structures than have appeared in previous VLA maps. In particular, the extended halo of overall diameter ~ 8 arc min (24 pc) around Sgr A^{35,36} is clearly represented in our 20-cm map. Some of the low-brightness details of this halo can be seen in Fig. 1.

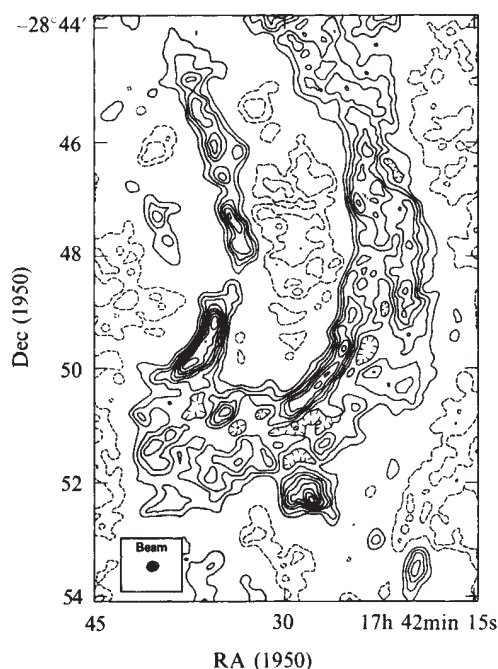


Fig. 2 Contour map of 6-cm emission from the arched filaments to the north of Sgr A. The contours are: ± 6.6 , ± 15.4 , 26.4, 39.5, 54.9, 72.5, 92.3, 114, and 138 mJy per beam area. The inset shows the contours of the beam response at 50, 70, and 90% of maximum. This map has been corrected for the primary beam response of the individual antennas; the full width at half power is about 8.6 arc min.

The two most striking characteristics of the Sgr A halo at 20 cm are: (1) the presence in the inner halo surrounding the Sgr A east shell of several radially-directed protrusions, and (2) the faint striations in the outer halo, which are all perpendicular to the galactic plane.

Some of the radially directed protrusions from Sgr A may be extensions of small-scale structure within the shell of Sgr A east, such as the northernmost arm of the spiral feature at the core of Sgr A west¹⁰. If this is so, any model for the nature of the spiral features (see ref. 12) is therefore constrained by their large extent.

On the large scale, the Sgr A halo is symmetrical about the galactic plane. The faint striations that can be discerned in the outer halo are systematically perpendicular to the galactic plane. Unfortunately, this kind of feature can also be an artefact of incompletely edited aperture synthesis data, so it must be regarded with caution until it can be confirmed by an independent observation. Further details on the Sgr A halo will be published separately.

Spectral index

We now briefly discuss a few of the more interesting results from the spectral index and polarization measurements.

Spectral index maps have been made by comparing 6- and 20-cm maps made with data in the same range of spatial frequencies, ensuring that emission at one frequency was being compared only with data at the other frequency which related to a similar scale size. Over most of the 6-cm fields, the spectrum is quite flat, with $\alpha \sim -0.2$ to 0.2 ($F_\nu \propto \nu^{-\alpha}$). Certain trends can be noticed, however. The spectral index tends to be consistently negative ($-0.2 \leq \alpha \leq 0$) in both the arched and the vertical filaments, whereas it is predominantly positive ($0 \leq \alpha \leq 0.2$) in G0.18–0.04. In any case, it seems that, except possibly for G0.18–0.04, one cannot use the spectral index information to differentiate clearly between thermal and non-thermal emission.

Over most of the region studied, any polarization of the emission is either weak or absent. However, at two places along

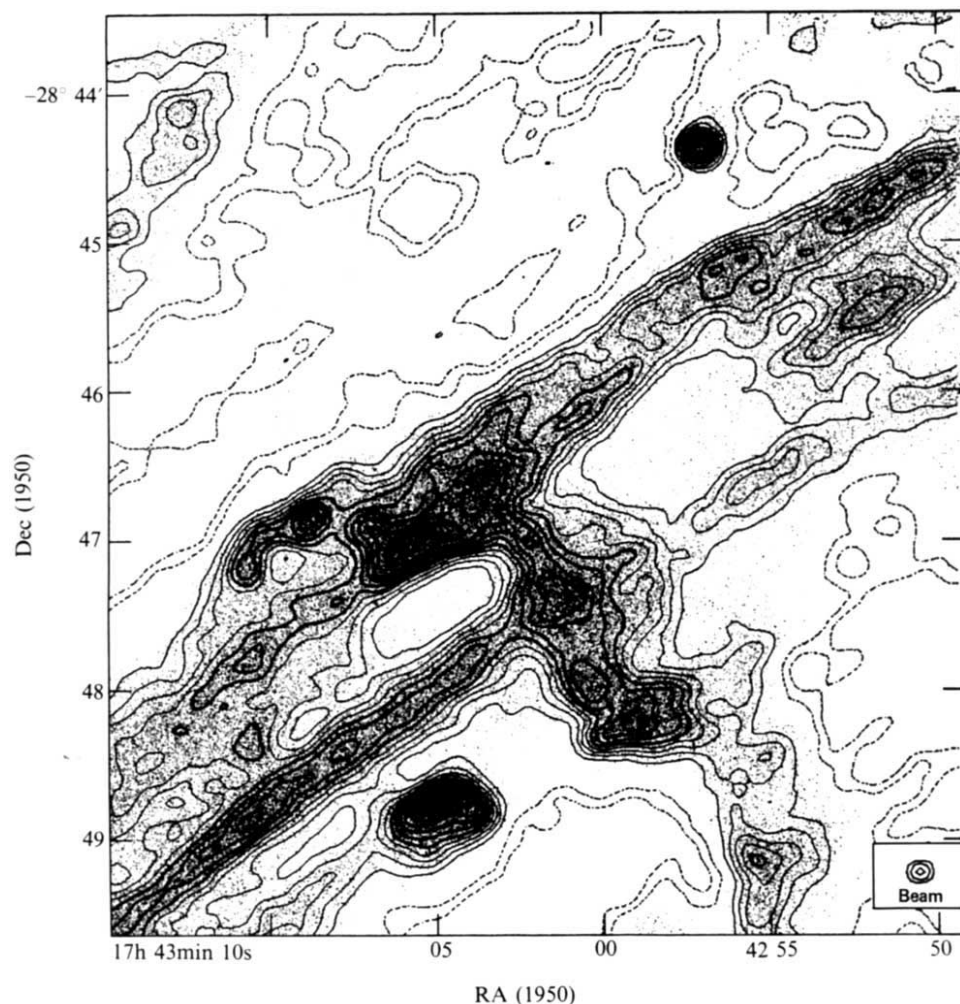


Fig. 3 Contour map of 6-cm emission from G0.18-0.04, where the vertical filaments cross the galactic plane. Contour levels are ± 3.9 , ± 9.1 , 15.7, 23.5, 32.6, 43.1, 54.8, 67.8, 82.2, 97.9 and 115 mJy per beam area. Other details are similar to Fig. 2.

the vertical system of filaments ($\alpha = 17^{\text{h}} 43^{\text{m}} 22^{\text{s}}$, $\delta = -28^{\circ} 51'$ and $\alpha = 17^{\text{h}} 43^{\text{m}} 16^{\text{s}}$, $\delta = -28^{\circ} 50'$), the polarization is relatively large, 15–25% at 6 cm. Curiously, these locations correspond roughly to the positions where the large helical structures cross the vertical filaments. At both of these locations, the electric vector of the polarized emission is roughly perpendicular to the filaments (although the importance of Faraday rotation has not been determined). Further south along the filaments from these two regions of maximum polarized intensity, polarized emission is present, although the percentage polarization is much smaller.

The observed polarization indicates that a substantial fraction of the observed radio emission is synchrotron radiation. However, because much of the observed radio emission is unpolarized, and because the radio spectrum is predominantly flat, one may conclude that there is an admixture of non-thermal emission and free-free emission and absorption in the system of filaments.

Implications

The filamentary radio structures in the Arc reveal a phenomenon which has not previously been considered for the galactic centre region. The scale of this phenomenon is large (± 150 pc, judging by the apparent extensions of the arc in the Altenhoff *et al.*³³ map), and the radio emission is continuous across this entire scale, so it is unlikely to be linked to localized occurrences such as star formation or supernova remnants^{24,25}. Furthermore, the radio arc is clearly a physically unified phenomenon, rather than the chance superposition of two or more unrelated sources of emission, as some have suggested^{17,19,25}. (Previous interferometer observations of the Arc region²⁵ were not sensitive to

continuous, large-scale structure, so the filaments reported here were perceived as numerous, separate H II regions.)

It must first be established that the Arc lies at the distance of the galactic centre, and is thus not a fortuitously superimposed foreground object. The radio structure in the Arc does not correspond to any phenomenon known to occur in the galactic disk. Filamentary radio structures exist (in supernova remnants, for example) but we know of no cases in which radio filaments are: (1) as nearly linear; (2) as even and unbroken on such a large angular scale; and (3) as parallel as the vertical filaments near the galactic centre. The polarization characteristics and the doubling of the filaments are also unknown elsewhere. The existence of the unique radio Arc is, therefore, easiest to contemplate if it occurs in a unique environment, such as the vicinity of the galactic centre. Moreover, the arched filaments appear to originate (or end) in the halo of Sgr A. That is, the placement and orientation of the Arc indicate a possible physical relationship between the Arc and the galactic nucleus. It is quite unlikely that this is a mere coincidence of projection.

The preceding two arguments are circumstantial. A more direct and conclusive argument can be made on the basis of the apparent relationship between the vertical filaments and the molecular cloud, M+0.11-0.08. We have argued that the vertical filaments are interacting with the radio structure G0.18-0.04. Other evidence^{13,15,16,37} indicates that G0.18-0.04 is itself interacting with the edge of the 40 km s⁻¹ molecular cloud (M+0.11-0.08). In particular, the cloud velocity, ~ 50 km s⁻¹, is similar to that of recombination line emission from G0.18-0.04 (ref. 19). Evidence that this molecular cloud lies near the galactic centre is fairly strong, and has been discussed elsewhere (ref. 37 and references therein). On the strength of these arguments,

we proceed with the presumption that the Arc represents a phenomenon occurring within the region of the galactic centre.

Could the apparently one-dimensional 'filaments' comprising the Arc actually be two-dimensional shock fronts (or ionized surfaces) that are limb-brightened by being viewed in projection from the side? The centre of curvature of the vertical filaments lies far from the galactic nucleus on the side opposite the Arc, near the radio source Sgr C, but because the shock velocity would increase with decreasing density and because the galactic centre region is probably a plane-stratified medium in which density declines with height, a shock could assume the shape of the curvature observed even if the origin of the shock is the galactic nucleus. The extent of the shock front would be enormous (40 pc) and the energy requirements correspondingly high, although perhaps not prohibitive. The major problem with a shock front model for the apparent filaments is their regularity and straightness. The inhomogeneities within the galactic centre region (the molecular cloud M + 0.11–0.08, for example) would be expected to introduce more breaks, kinks and general disorder into a shock front passing over them than are observed in the filaments making up the Arc. Moreover, the outer helical structure, whether apparent or real, would be very difficult to fit into a picture of the Arc as a shock front.

We conclude that the geometry of the Arc is essentially linear, and that it is controlled by magnetic phenomena. This observation therefore provides the first indication of a substantial poloidal component to the magnetic field near the galactic centre. The substantial degree of polarization observed at a few positions along the filaments is most readily explained as synchrotron radiation and, if Faraday rotation is negligible, the mean orientation of the position angle of the polarized *E*-vector at those positions thereby implies that the magnetic field lines are parallel to the long axis of the filaments, as might be expected. The resolution of the Arc into a sheath of filaments might then be accounted for in terms of filamentation instabilities³⁸ perhaps analogous to those occurring in solar prominences.

If the helicity of some of the structures that were observed can be verified, this would strongly support the hypothesis that magnetic fields are important in the Arc. Indeed, the helical geometry might be revealing the presence of a nearly force-free magnetic field configuration. However, although the magnetic lines of force in a cylindrically symmetrical force-free field are helically wound about the axis of symmetry^{39,40}, the plasma characteristics, and thus the emergent radio emission, should be cylindrically symmetrical, unless the force-free system has undergone filamentation instabilities.

At the projected distance of the vertical filaments from the galactic centre, the period for circular rotation is only $\sim 10^6$ yr.

Also, the filaments are not within the region of solid-body rotation. Therefore, unless the lifetime of the filamentary structure is much shorter than 10^6 yr, one might expect differential rotation to deform it and wrap it into a primarily azimuthal structure. However, the apparent curvature of the vertical filaments is very similar, if not essentially identical, to that needed to keep all points on the filament rotating at a constant angular velocity (based on the mass distribution given by Oort¹). This conspiracy would permit the filaments to have an arbitrarily long lifetime. This comment does not apply to the arched filaments, which must be rapidly evolving features.

In view of our results, one may question whether the formation of massive stars is occurring at an important rate in the galactic centre region, as has often been assumed. Indeed, when the large quantity of dense molecular gas within a few hundred parsecs of the galactic centre is considered^{37,41,42}, then, except for Sgr B2, and perhaps one or two other sites, there is a definite lack of evidence that the rate of massive star formation per unit mass of gas is comparable to that in the galactic disk. This claim is based on a relative absence of strong H₂O masers⁴³ and an apparently low rate of occurrence of compact H II regions such as might be produced around O stars.

The emission associated with G0.18–0.04 was suggested by Pauls and Mezger¹⁹ to be a region of star formation. Our results do not contradict this suggestion, but they provide a viable alternative to OB stars as the source of ionization. As G0.18–0.04 appears to be interacting with the sheath of vertical filaments, we suggest that the impact of the particles within the relativistic plasma of the filaments on ambient gas in the galactic plane might be responsible for the ionization. Indeed, according to the arguments above, the ambient gas is present at the right location in the form of the '40 km s⁻¹' molecular cloud. A higher resolution study of the region surrounding G0.18–0.04, such as is possible with a larger configuration of the VLA, and with the new generation of millimetre telescopes, should make it possible to test the hypothesis that this object results from the impingement of a magnetically confined, relativistic plasma on cool molecular gas.

We thank many NRAO personnel, particularly Bill Cotton, Pat Crane, Tim Cornwell, Robert Brown, Harvey Liszt, Peggy Perley, Barry Turner and Eric Greisen for helpful suggestions or for passing on their expertise in data analysis. We also thank John Bally and K. Y. Lo for access to computational facilities, Kevin Prendergast, Ian Gatley and Roger Blandford for discussions, and R. J. Havlen for travel support. This research was supported by NSF grant AST81-14716 to Columbia University. The NRAO is operated by Associated Universities, Inc., under contract with the NSF.

Received 6 April; accepted 14 June 1984.

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Tissue-specific activation of a cloned α -fetoprotein gene during differentiation of a transfected embryonal carcinoma cell line

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To identify cis-acting DNA elements involved in the activation of the α -fetoprotein gene during differentiation, modified copies of the gene were introduced into murine F9 embryonal carcinoma cells. The differentiation of the transformants to either parietal or visceral endoderm was accompanied by induction of the exogenous template in a manner qualitatively, but not quantitatively, identical to that of the endogenous α -fetoprotein gene.

IN the mouse the development of the extra-embryonic yolk sac begins early in embryogenesis. Towards the end of the fourth day after fertilization the progenitor tissue, primary endoderm, segregates from the inner cell mass^{1,2}. By day 7 the parietal and visceral endodermal layers are evident³ and constitute the distal and proximal cell walls of the yolk sac, respectively. These latter two endodermal cell types can be distinguished by a variety of morphological criteria and biochemical markers⁴. Parietal endoderm cells rest on a thick basement membrane, termed Reichert's membrane, and are comparatively enriched in the synthesis and secretion of the membrane components collagen type IV, laminin, entactin and heparin sulphate proteoglycan⁵⁻⁷. Visceral endoderm cells are associated with a thin basement membrane and are characterized by the synthesis of α -fetoprotein (AFP)⁸. AFP cannot be detected in parietal endoderm or in any of the progenitor tissues of the early mouse embryo. Its synthesis in visceral endoderm can be detected by day 7 of development⁸ and by day 15.5 its mRNA accounts for 20% of the total poly(A)⁺ RNA in visceral endoderm cells⁹.

To understand the mechanisms underlying gene activation during development, we have studied the activation of the AFP gene in visceral endoderm. The study of early mouse embryogenesis has been greatly facilitated by the establishment of embryonal carcinoma (EC) cell lines derived from the stem cells of teratocarcinomas¹⁰. EC cells possess many properties of the ectodermal cells of the inner cell mass¹¹ and cell lines exist that are capable of differentiating in culture to a variety of tissue types, including extra-embryonic endoderm. One such line, F9, has been well characterized and has a very low frequency of spontaneous differentiation¹². Strickland and co-workers^{13,14} have shown that after treatment with retinoic acid and cyclic AMP, a monolayer of F9 EC cells will differentiate to a cell closely resembling parietal endoderm. If F9 EC cells are treated instead with retinoic acid alone and are permitted to aggregate, most of the cells on the outer surface of the aggregate acquire a phenotype very similar to that of visceral endoderm, and consequently synthesize AFP¹⁵. These differentiated aggregates have been termed embryoid bodies because of their structural resemblance to early blastocysts¹⁶.

Transformation of F9 cells

We have used this system to identify any *cis*-acting DNA elements within the AFP genomic locus that are required for the activation of the AFP gene on differentiation in culture. To approach this question, we have introduced, via DNA transformation, modified copies of the mouse AFP gene into F9 EC cells and examined whether the pattern of expression of the AFP gene in the F9 stem and differentiated cells could be reproduced from the exogenous AFP templates.

Stable transformants were established by co-transformation of an F9 TK⁻ (thymidine kinase-negative) EC cell line with

unlinked plasmids containing the 3.4-kilobase (kb) *Bam*HI fragment of the herpes virus thymidine kinase gene¹⁷ and an AFP minigene termed YZE (Fig. 1). The AFP minigene was constructed by joining the two contiguous 5' AFP genomic *Eco*RI fragments Y and Z to a 3' distal *Eco*RI fragment, E^{18,19}, resulting in a five-coding block gene containing 14 kilobase pairs of 5' flanking DNA, the first three and the last two coding blocks of the AFP gene and 0.4 kb of 3' flanking DNA. This construction contains all of the DNA between the 3' terminus of the albumin gene and the 5' terminus of the AFP gene, and thus will test whether putative *cis*-acting DNA elements required for proper AFP gene regulation are located in this intergenic region. The mature transcript from the YZE minigene should be approximately 600 bases long and therefore distinguishable from the

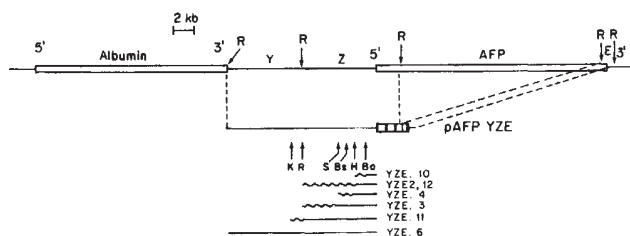
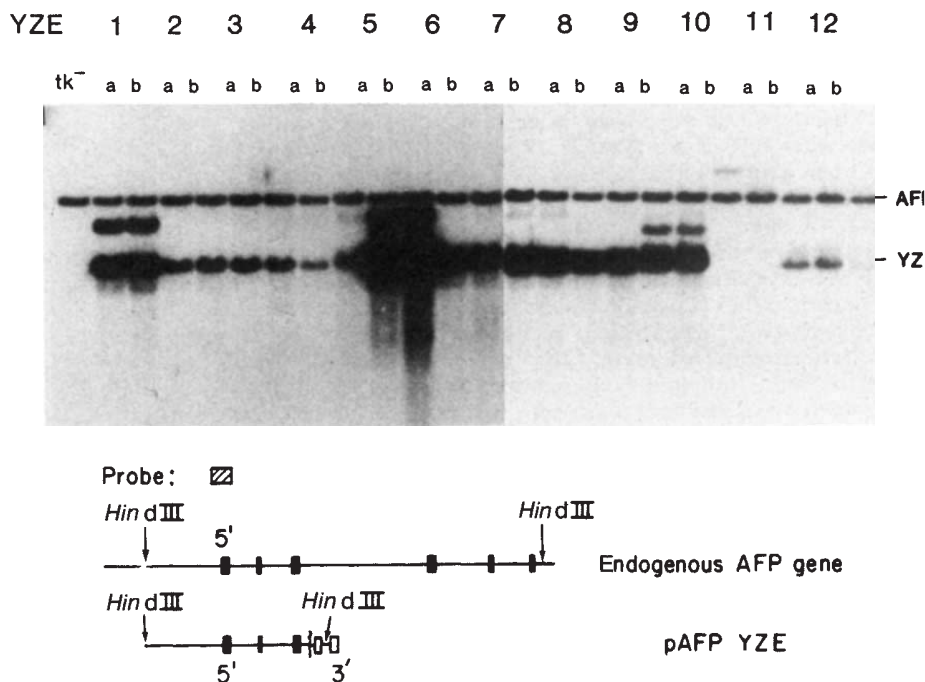


Fig. 1 Structure of pAFPYZE and its configuration in several single-copy F9 transformants. The minigene pAFPYZE was constructed in two stages using three subgenomic clones of the albumin-AFP gene loci, shown at the top of the figure. The *Eco*RI Y fragment contains 7 kb of 5' distal flanking DNA, the *Eco*RI Z fragment contains 7 kb of 5' proximal flanking DNA and the first three exons of the AFP gene, and the E fragment contains the last two exons, plus 0.4 kb of 3' flanking DNA. The structure of the resulting 5-exon minigene is illustrated in the centre; the line drawings underneath illustrate the approximate breakpoint in the 5' flanking regions of the integrated YZE DNA in the low-copy transformants. The straight line represents sequences contained in the transformants and the wavy line represents the region where the breakpoint occurs. The enzyme digests used to map these sites are: Ba, *Bam*HI; H, *Hind*III; Bs, *Bst*EII; S, *Sma*I; R *Eco*RI; K, *Kpn*I.

Methods: To construct pAFPYZE, the 1.1-kb *Eco*RI E fragment of pAFP7E¹⁸ was joined to the 9.8-kb *Eco*RI Z fragment of pAFP14Z¹⁸. pAFP7E was digested to completion with *Eco*RI and ligated at equimolar concentrations to a partial *Eco*RI digest of pAFP14Z at a final concentration of 25 μ g ml⁻¹. The ligation products were used to transform HB101 cells and ampicillin-resistant colonies were screened by colony hybridization for plasmids in which the 1.1-kb AFP7E *Eco*RI fragment was inserted in pAFP14Z. Proper orientation of the fragment was confirmed by restriction enzyme analysis and the resultant recombinant is termed pAFPZE. Then, the 7.0-kb *Eco*RI Y fragment of pAFP15Y¹⁹ was joined to AFPZE by the same procedures as described above.

Fig. 2 Genomic DNA blots of YZE transformants. YZE-specific DNA sequences were detected in genomic DNA from 12 transformants using a hybridization probe specific for the first coding block of the AFP gene. HAT-resistant clones were grown under selective pressure (lanes a) or in the absence of selective pressure for over 50 generations (lanes b). The diagram below the figure illustrates the expected 10-kb fragment from the endogenous AFP gene and the 5-kb fragment from YZE DNA.

Methods: F9 YZE transformants were obtained by co-transformation of F9 TK⁻ cells (given by B. Knowles and D. Solter) with two plasmids containing the herpesvirus *tk* gene (3.4-kb *Bam*HI fragment¹⁷) and the AFP YZE minigene. F9 TK⁻ cells (10^6 cells per 10 cm plate) in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS; Armour) were incubated with 10 μ g of pTK DNA and 10 μ g of pAFPYZE DNA in the presence of $\text{Ca}_3(\text{PO}_4)_2$ (ref. 44). After 12 h the medium was removed, the monolayer cultures were treated with 15% glycerol in 1 $\times$ Hank's buffered saline for 3 min at room temperature, and maintained in DMEM plus 10% FCS for 24–36 h. At this time HAT selective medium (DMEM, 10% FCS, 15 μ g ml⁻¹ hypoxanthine, 1 μ g ml⁻¹ aminopterin, 10 μ g ml⁻¹ thymidine) was added; 2–3 weeks later, HAT-resistant colonies were picked and expanded for DNA analysis. DNA was isolated by resuspending the cell pellets containing $\sim 5 \times 10^6$ cells in 1 ml of 0.3 M Tris-HCl (pH 8.0), 0.1 M NaCl, 0.2 M sucrose, 0.05 M EDTA and then adding 0.25 ml of 0.5 M Tris-HCl (pH 9.2), 0.25 M EDTA and 2.5% SDS to lyse the cells. The cell lysate was incubated at 60°C for 15 min then treated with 100 μ g ml⁻¹ proteinase K for 12–15 h. The lysate was brought to 0.5 M NaCl, extracted with equal volumes of phenol, chloroform and ether and nucleic acids were precipitated by adding an equal volume of ethanol at room temperature. The DNA was spooled, washed once with 70% ethanol at room temperature and resuspended in 10 mM Tris-HCl (pH 7.5). To detect YZE specific DNA, 10 μ g of the DNA samples were digested with a 3–5-fold excess of *Hind*III and electrophoresed on a 1% agarose gel. The DNA was transferred to nitrocellulose⁴⁵ and hybridized with a labelled probe that spans the first AFP coding block.



endogenous AFP mRNA, ~ 2.2 kb long. The major assumptions in the use of the YZE minigene are that such a construct can generate a stable transcript and that by deleting the internal regions of the AFP gene together with proximal 3' sequences, important regulatory elements have not been removed. To test the first assumption, the five coding block minigene was inserted into the simian virus 40 (SV40)/pBR322 vector, pSV2 (ref. 20), and transfected into HeLa cells. A stable 600 base-long transcript consisting of correctly initiated, spliced and polyadenylated RNA was detected and used throughout as a standard for YZE mRNA (unpublished data).

The F9 TK⁻ EC cells were co-transformed with pYZE and the herpes virus *tk* DNA as described in Fig. 2 legend. Hypoxanthine-aminopterin-thymidine (HAT)-resistant colonies were obtained at a frequency of 1–10 colonies per μ g DNA per 10^6 cells. The colonies were individually expanded under selective pressure and screened by restriction enzyme analysis for the presence of YZE-specific DNA sequences. Genomic DNA from the expanded cultures was hybridized after electrophoresis to a probe that spans the first AFP coding block. This probe hybridizes to a 10-kb *Hind*III fragment from the endogenous AFP gene and a 5-kb fragment from the YZE minigene, due to the presence of a *Hind*III site in YZE in the 14th intervening sequence (Fig. 2). The screen identified a total of 11 transformants that contained an intact 5-kb YZE *Hind*III fragment (Fig. 2, lanes labelled a). An additional transformant (YZE.10) had a rearranged *Hind*III fragment.

The copy number of the YZE DNA varied between transformants, from 1 to >120 copies per cell. This remained constant for 11 of the 12 transformants when maintained in nonselective conditions, indicating that the YZE DNA was stably integrated into the genome (compare lanes a and b for each transformant in Fig. 2). YZE.10 apparently represents an unstable transformant as the signal corresponding to the YZE-specific fragment was greatly diminished during growth in nonselective medium.

Induction of YZE mRNA

We next determined the qualitative and quantitative pattern of expression from the YZE templates, relative to the endogenous gene, in the undifferentiated and differentiated cultures of each transformant in Fig. 2. Total poly(A)⁺ RNA was isolated from undifferentiated EC cell monolayers and 15-day embryoid body cultures of each transformant. After electrophoresis, the RNA was hybridized to the first AFP coding block probe described in Fig. 2.

Figure 3a shows that authentic AFP mRNA was detected only in the embryoid body RNA (V lanes) for each transformant, confirming the presence of visceral endoderm in the cell aggregates. RNA species of the appropriate size for fully processed YZE mRNA (arrow in Fig. 3) were detected at relatively low levels in YZE.7,8,9,1 and 5 embryoid bodies but were absent from the corresponding undifferentiated EC cells. As for YZE.12 and 6 and the YZE transformants not shown in Fig. 3, no significant amounts of RNA species corresponding to the predicted size of YZE mRNA were detected in either the EC cells or embryoid bodies. In many of the transformants, RNA species of higher molecular weight were detected in both EC and embryoid body cultures; these will be discussed below. Finally, in YZE.11, a transcript larger than that expected for YZE mRNA was expressed constitutively.

To ensure that the 600-base transcript does not merely represent a size class of nonspecific degradation products of AFP mRNA, several of the RNA samples in Fig. 3a were hybridized with an AFP cDNA probe specific for internal coding blocks not represented in the YZE minigene. As shown in Fig. 3b, this probe hybridized with the endogenous AFP mRNA but not with the 600-base RNA.

Characterization of YZE mRNA

To provide additional proof that the 600-base RNA is YZE mRNA, we performed an S₁ nuclease hybridization assay that

would distinguish YZE mRNA from AFP mRNA. Both transcripts should have the same 5' and 3' termini but differ in that YZE mRNA should lack sequences between the 3rd and 14th AFP coding blocks. Therefore, a DNA probe that spans the third and fourth coding blocks would be fully protected from S_1 nuclease when hybridized to AFP mRNA but only partially protected when hybridized to YZE mRNA (see diagram in Fig. 4). The first lane of Fig. 4 contains the DNA fragment protected by YZE mRNA isolated after transient expression in HeLa cells and this serves as a standard for the 91-base S_1 -protected fragment. The 91-base fragment was also observed with RNA samples from embryoid body cultures of the YZE transformants that were positive for YZE mRNA by gel analysis (Fig. 3).

Unexpectedly, significant levels of the 91-base fragment were also obtained from embryoid body cultures of YZE transformants in which no YZE mRNA was detected (YZE.6,10,2 and to a lesser extent YZE.3). In addition, the fragment was also detected using RNA from several undifferentiated EC cell cultures, none of which contained YZE mRNA by gel analysis

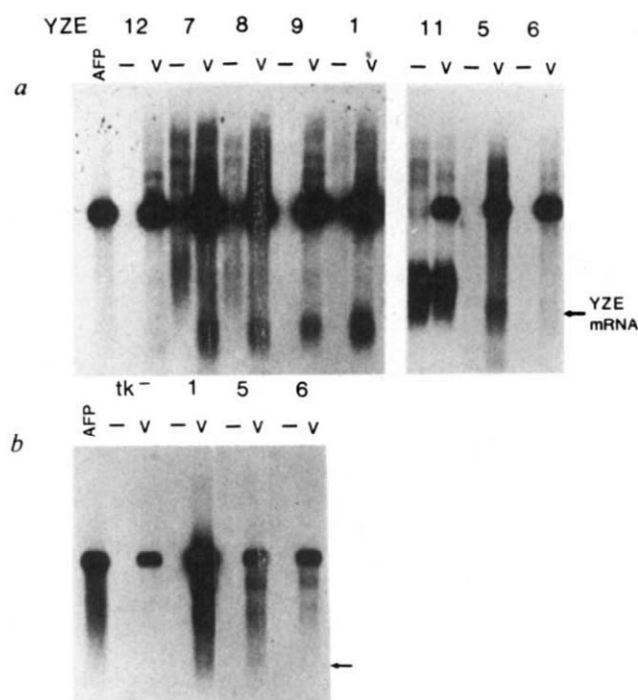


Fig. 3 Analysis of AFP and YZE mRNAs in EC cell and embryoid body cultures of YZE transformants: 7 μ g of poly(A)⁺ RNA from EC cells (-lanes) or embryoid bodies (V lanes) from the indicated transformants were electrophoresed through an agarose gel and transferred to nitrocellulose filters. *a*, The nitrocellulose filters were hybridized to a labelled probe that spans the first AFP coding block (described in Fig. 2). *b*, The nitrocellulose filters were hybridized with the labelled *Pst*I insert of the cDNA clone, pAFP2 (ref. 46). This sequence contains AFP coding blocks 7-13. The AFP lanes in *a* and *b* correspond to 20 ng of poly(A)⁺ fetal mouse liver RNA. All autoradiograms were exposed for 48 h.

Methods: Undifferentiated EC cell cultures were maintained as monolayers in tissue culture flasks in DMEM with 10% FCS. Embryoid body cultures were established as described previously^{15,23} by seeding 1×10^6 EC cells in the presence of 7.5×10^{-8} M all-trans retinoic acid (Eastman) in 10 cm bacteriological Petri dishes, and collected after 15 days. Total cell RNA was prepared by the hot phenol method⁴⁷ and enriched for poly(A)⁺ RNA by oligo (dT)-cellulose chromatography⁴⁸. Poly(A)⁺ RNAs were denatured at 55°C for 15 min in 60% formamide, 6% formaldehyde and 1 M MOPS buffer, pH 7.0 (20 mM MOPS (morpholine propanesulphonic acid), 5 mM sodium acetate, 1 mM EDTA), electrophoresed in a 6% formaldehyde, 1.5% agarose gel in 1 M MOPS buffer and the RNA was transferred to nitrocellulose in $20 \times$ SSC⁴⁹ (1 M SSC = 0.15 M NaCl, 0.015 M sodium citrate).

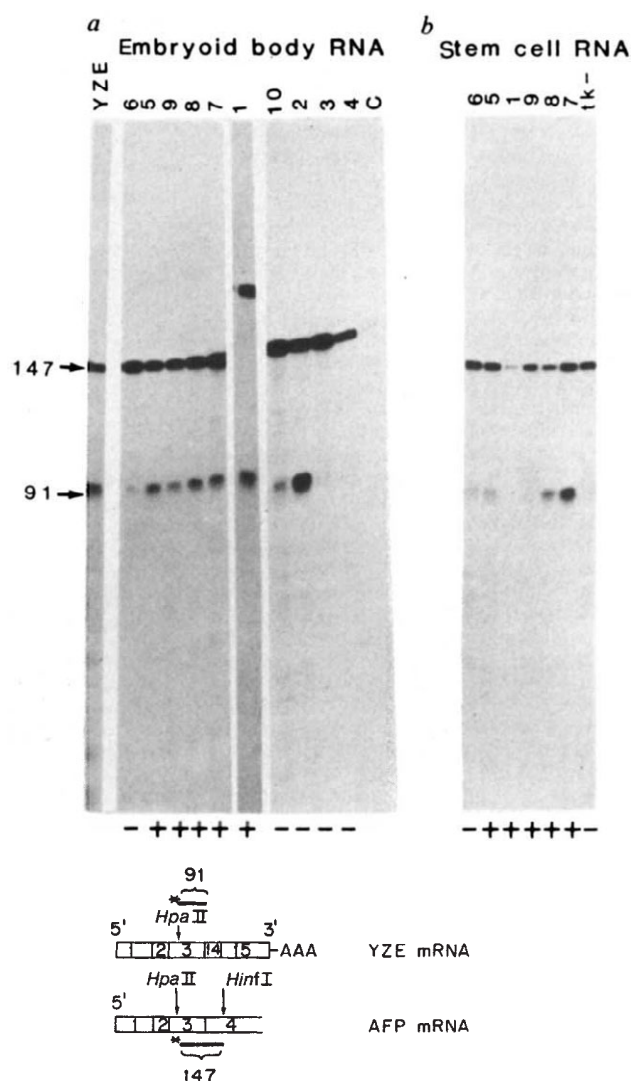


Fig. 4 S_1 nuclease hybridization assay to distinguish YZE mRNA from AFP mRNA. The S_1 hybridization probe used in this experiment is a 3' end-labelled 147-nucleotide *Hpa*II-*Hinf*I fragment derived from an AFP cDNA clone (pAFP1)⁴⁶ and contains sequences from both the third and fourth AFP coding blocks. *a*, S_1 protection of 20 μ g of poly(A)⁺ RNA from the indicated embryoid body cultures. The lane labelled C represents a tRNA control. *b*, S_1 protection of 20 μ g of poly(A)⁺ RNA from the indicated EC cell cultures. The symbols below the lanes indicate those cell lines for which YZE mRNA was detected (+) or not detected (-) by Northern blot analysis (see Fig. 3).

Methods: To prepare the probe, 25 μ g of pAFP1 DNA were digested with *Hpa*II and 3' end-labelled by resuspending the DNA in a total volume of 30 μ l containing 34 mM Tris-HCl (pH 8.3), 50 mM NaCl, 6 mM MgCl₂, 5 mM dithiothreitol and 70 μ Ci [α -³²P]dCTP (800 Ci mmol; NEN), then treating the mixture with 4 units of AMV reverse transcriptase (Life Sciences) for 1 h at 37°C. The DNA was further digested with *Hinf*I and the 147-bp strand-separated *Hpa*II-*Hinf*I fragment was isolated on a 5% polyacrylamide gel (acrylamide/bisacrylamide, 62.5:1) in 1 M TBE buffer (90 mM Tris-borate pH 8.3, 4 mM EDTA) and recovered by electroelution. S_1 hybridization assays⁵⁰ were done as described previously²⁰ using 25 ng of the end-labelled *Hpa*II-*Hinf*I fragment. The YZE transformant samples contained 20 μ g of poly(A)⁺ RNA per hybridization mixture and the HeLa cell control RNA samples contained 10 ng of poly(A)⁺ RNA isolated after transient expression of YZE inserted in an SV40/pBR322 vector, pSV₂ (ref. 20) (lane YZE). The denatured fragments were electrophoresed on a 7 M urea, 7.5% polyacrylamide gel in 1 M TBE buffer. Molecular weight markers were single-stranded ϕ X174 DNA (Miles) digested with *Hae*III and end-labelled with [γ -³²P]ATP (1,000 Ci mmol⁻¹, NEN) and polynucleotide kinase (see Fig. 5).

(Fig. 4b). One explanation for the detection of this fragment in the absence of YZE mRNA is that transcription is initiating at sites other than the AFP cap site and that these readthrough transcripts are being spliced at the 3' border of the third AFP coding block. To address this possibility, YZE transcription in several undifferentiated EC cell cultures was analysed by S_1 nuclease hybridization assays using the 5'-specific probe described in Fig. 5 legend. As the AFP mRNA is not expressed in EC cells (see Fig. 3), this assay will determine if properly initiated YZE transcripts are present before differentiation. Figure 5a shows that no accurately initiated AFP transcripts were detected in any of the RNA samples isolated from the undifferentiated YZE EC cells at a sensitivity equal to that of the internal splice S_1 assay in Fig. 4.

However, an S_1 -protected fragment that maps to position -33 was detected in several samples. Transcripts defined by the -33 S_1 nuclease-protected fragments have also been detected in HeLa transient expression experiments using a variety of AFP minigene constructs inserted in SV40/pBR322 vectors²⁰. The simplest interpretation of these results (and the one we favour) is that the -33 fragments are the result of a cryptic 3' splice junction at this position—that is, a relatively low level of constitutive readthrough transcription is occurring over the YZE locus in many of the YZE transformants. The initiation sites of the transcripts are unknown but at least a portion of them are using the cryptic splice site at position -33 and the 3' splice junction of the third AFP coding block. By standardizing the signals obtained from RNA isolated from the YZE transformants to those from the RNA controls, it seems that the levels of the constitutive transcripts are not significantly altered on differentiation. Further, the presence of this class of transcripts may explain the higher molecular weight background signal observed in the RNA blots in Fig. 3.

A particularly striking example of this phenomenon is seen with YZE.11. In this transformant an RNA species larger than YZE mRNA was detected at high levels in both the undifferentiated EC cell and embryoid body cultures (Fig. 3). This transcript is polyadenylated and does not hybridize with the internal cDNA probe (data not shown). When poly(A)⁺ RNA isolated from YZE.11 undifferentiated EC cells was analysed by the 5'-specific S_1 nuclease hybridization assay, a set of protected fragments mapped to position -33. Only a splice at this site, rather than transcription initiation, could account for the size discrepancy between the gel data (Fig. 3) in which the transcript appeared to be approximately 200 bases larger than expected and the S_1 nuclease protection assay (Fig. 5b) in which a fragment only 33 bases larger than expected was observed. The initiation site of this transcript has not been identified.

Due to the significant level of nonspecific transcription over the YZE loci, we wanted to confirm directly that the transcripts identified as YZE mRNA in Fig. 3 were initiated at the proper AFP cap site. YZE mRNA from embryoid body cultures of YZE.1 was purified from a denaturing agarose gel and used in an S_1 hybridization assay with the 5'-specific DNA probe described in Fig. 5 legend. The radiolabelled fragments protected from S_1 nuclease digestion were resolved on a denaturing polyacrylamide gel and the results in Fig. 5c show that the eluted RNA has the same 5' terminus as AFP mRNA and the protected 91-base fragment that is characteristic of YZE mRNA but not AFP mRNA (data not shown).

Tissue specificity of YZE mRNA expression

The tissue specificity of expression from the exogenous YZE templates was investigated by comparing visceral and parietal endoderm derived from the YZE EC cells. Endoderm cultures were established by treating EC cell monolayers with retinoic acid and cyclic AMP (parietal) or allowing the EC cells to aggregate in the presence of retinoic acid for 15 days (visceral). Differentiation to parietal endoderm was confirmed by morphology^{14,21} and by induction of the major histocompatibility antigen (H-2) mRNA^{22,23} (data not shown). Poly(A)⁺ RNA was

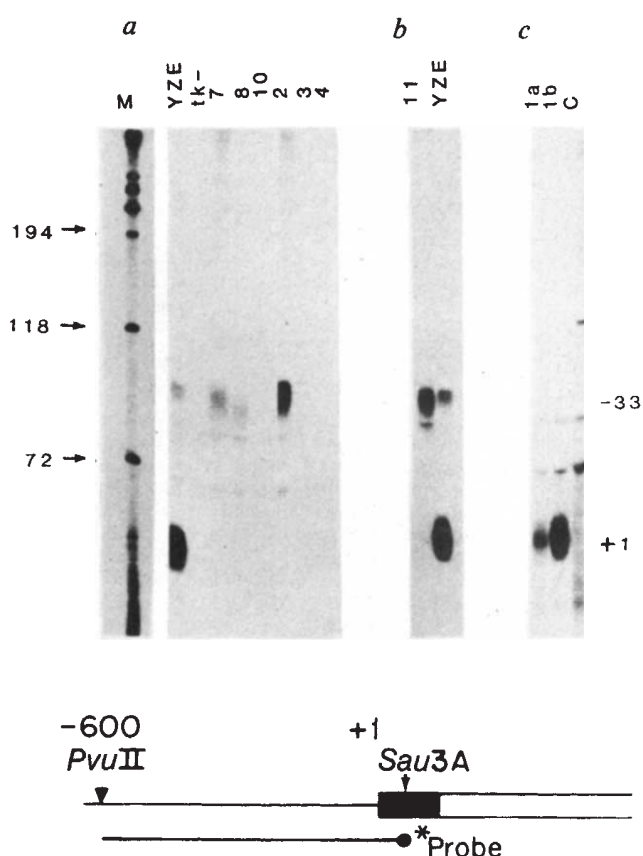


Fig. 5 S_1 hybridization assay to map the readthrough transcripts in the undifferentiated EC cell transformants. The hybridization probe is the *Sau3A*-*PvuII* 5' end-labelled probe described previously²⁰ and illustrated in the figure. The hybridization and S_1 digestion conditions were as described previously²⁰. Lane M, single-stranded ϕ X174 DNA digested with *HaeIII* and end-labelled with [γ -³²P]ATP and polynucleotide kinase. **a:** Lane YZE, S_1 protection of 200 ng of poly(A)⁺ RNA from HeLa cells transfected with YZE/SV40/pBR322 DNA²⁰. The subsequent lanes are 20 μ g of poly(A)⁺ RNA from the indicated undifferentiated EC cells. **b:** S_1 protection of 10 μ g poly(A)⁺ RNA from undifferentiated EC cells of YZE.11; and 200 ng of poly(A)⁺ RNA from HeLa cells transfected with YZE/SV40/pBR322 DNA (lane YZE). **c:** Lane 1a, size-selected poly(A)⁺ RNA from embryoid body cultures of YZE.1; lane 1b, 10 μ g unfractionated poly(A)⁺ RNA from embryoid body cultures of YZE.1; lane C, tRNA control.

extracted from each of the tissue types, and after electrophoresis, hybridized with the first AFP coding block probe (Fig. 6). As described in Fig. 3, induction of YZE mRNA was detected on formation of visceral endoderm for YZE.7,8,9,1 and 5. When RNA extracted from parietal cultures of these transformants was analysed, neither the endogenous AFP nor YZE mRNAs were detected. Thus, we conclude that the qualitative pattern of expression from the endogenous AFP gene and the exogenous YZE genes is identical in the three tissue types examined. The constitutively expressed aberrant transcript in YZE.11 can also be detected in parietal endoderm at levels comparable to those found in visceral and EC cell cultures.

To analyse the specificity of YZE mRNA induction with regard to other transcripts encoded by the input DNA, levels of the herpesvirus *tk* transcripts were measured in the EC and embryoid body cultures of each of the 12 YZE transformants that had been passaged for over 50 generations in the absence of selective pressure. Except for one transformant, YZE.2, in which a slight increase of *tk* mRNA was observed, the level of *tk* mRNA remained constant, or decreased on formation of the embryoid body (data not shown).

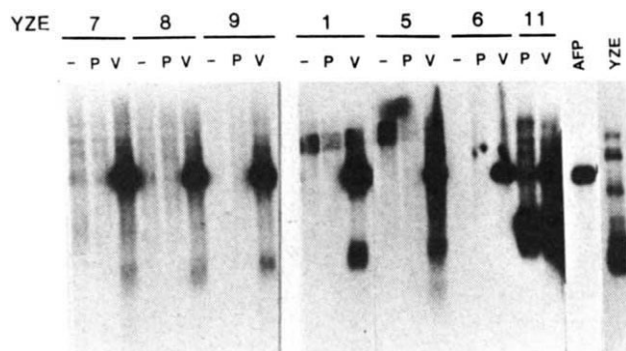


Fig. 6 Analysis of AFP mRNA and YZE mRNA expression in parietal endoderm cultures. 7 µg of poly(A)⁺ RNA isolated from EC cells (-), parietal (P) and 15-day embryoid body (V) cultures were electrophoresed on denaturing agarose gels as described in Fig. 3 legend, transferred to nitrocellulose filters and hybridized with a nick-translated probe that spans the first AFP coding block. The AFP lane contains 20 ng of poly(A)⁺ fetal mouse liver RNA and the YZE lane contains 0.5 µg of poly(A)⁺ HeLa RNA isolated after transient expression of YZE/SV40/pBR322 DNA. Exposure time was 3 h for the YZE autoradiogram and 48 h for all others. **Methods:** Parietal endoderm cultures were established as described previously^{14,23} from EC cell cultures of the indicated YZE transformants. The cells were collected by trypsinization 5 days after seeding, then RNA was extracted by the hot phenol method and enriched for poly(A)⁺ RNA by oligo(dT)-cellulose chromatography. The undifferentiated EC cells and 15-day embryoid body cultures were established as described in Fig. 3 legend.

Temporal expression of YZE mRNA

It has been suggested that the production of visceral endoderm in the outer layer of aggregating F9 cells occurs as an orderly progression of multiple events²⁴ and that maximum expression of AFP requires the production of an organized epithelium properly aligned on a thin basement membrane²⁵. A time course for the induction of AFP expression reveals that the levels of AFP mRNA increase continually for at least 17 days after aggregation, with the major accumulation occurring after 7 days²³. In contrast, other endodermal markers such as H-2 mRNA and albumin mRNA increase until day 5, after which they remain relatively constant²³. The very low levels of YZE mRNA in all the positive transformants (Figs 3, 6) raised the issue of whether one or more of the stages in AFP mRNA induction was not occurring properly. Therefore, we examined the time course for induction of YZE mRNA in two transformants. Total poly(A)⁺ RNAs from YZE.9 and YZE.1 were extracted from embryoid body aggregates at various times after seeding in the presence of retinoic acid. Figure 7 shows that AFP mRNA was detectable at very low levels by day 5 in both transformants, increased dramatically between days 7 and 9 and continued to increase up to day 15. YZE mRNA was barely detectable by day 7 in both transformants, and increased only 2–3-fold by day 15. During this same period, densitometric scans revealed that AFP mRNA increased ~100-fold in YZE.1 and >500-fold in YZE.9 (see the bottom panel in Fig. 7 for the short exposure of the autoradiogram). Hybridization of the washed filters with a mouse α -actin probe confirmed that each lane contained equivalent amounts of RNA (data not shown).

Gene copy number and YZE inducibility

A comparison of Figs 2 and 3 reveals that the five transformants capable of inducing YZE mRNA were all multiple-copy DNA integrants (YZE.1, 5, 7, 8 and 9). Only one of the non-inducers, YZE.6, contained multiple inserts. Although other possibilities exist, it may be that the single-copy integrants have not retained sufficient co-linear DNA in the critical region to confer inducibility. Thus, the extent of 5' flanking DNA in each of these trans-

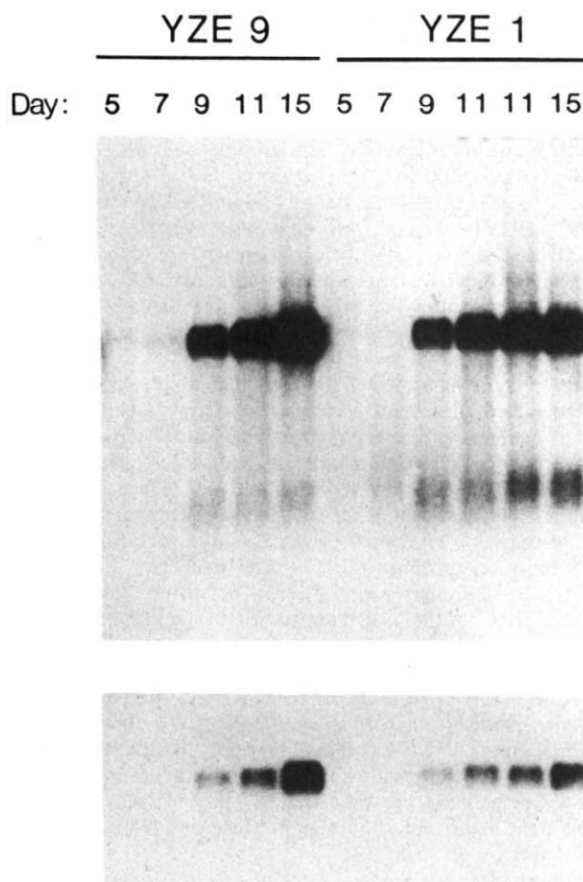


Fig. 7 Time course of AFP mRNA and YZE mRNA production during embryoid body formation. Embryoid body cultures of YZE.9 and YZE.1 transformants were established as described in Fig. 3 legend. At the indicated days after seeding in bacteriological plates in the presence of retinoic acid, cultures were collected and RNA was extracted by the hot phenol method. For each sample, 7 µg of poly(A)⁺ RNA were electrophoresed on a denaturing agarose gel, transferred to nitrocellulose filters and hybridized with a labelled probe that spans the first AFP coding block (see Fig. 3). The day 5–15 samples for YZE.9 were collected from one series of embryoid body cultures. For YZE.1, the day 5 to the first of the day 11 samples were collected from one series of embryoid body cultures, and the second day 11 and day 15 samples were collected from a second series of embryoid body cultures. The top panel is a 48-h exposure and the bottom panel a 3-h exposure of the same nitrocellulose filter.

formants was examined by polymorphic restriction enzyme digestions²⁶. The results showed that all of the multiple-copy transformants (YZE.1, 5, 6, 7, 8 and 9) contained multiple copies of intact YZE insertions. The low-copy transformants YZE.2, 3, 4 and 12 had intact copies of the structural gene but had 5' flanking DNA breakpoints located between map positions -7,400 and -2,000, respectively (see Fig. 1). The 5' junction site for YZE.11 lay between map positions -8,400 and -7,400, respectively. Thus, the only non-inducer with an intact integrated YZE gene was YZE.6.

Conclusions

The introduction of cloned genes into differentiated mammalian cells in culture has been widely used to study regulatory mechanisms of gene expression. Much of this work has focused on the induction of expression from transfected genes in response to external modulating signals^{27–41}. In these experiments, the recipient cells were fully committed to a specific developmental pathway. The F9 EC cell, in contrast, is a multipotent stem cell

capable of differentiation into two different endoderm cells as well as into adrenergic neurone-like cells⁴². Before differentiation the AFP gene is completely silent in these cells, and is activated selectively on formation of visceral endoderm¹⁵. To study this activation event, the AFP minigene promoter had to be inactive in the EC cell, a condition that held in all the transformants examined. However, while we detected no authentic YZE mRNA in the undifferentiated cells, transcription was clearly occurring within the exogenous DNA, albeit not at the AFP promoter. There was no obvious correlation between this constitutive transcription through the locus and the subsequent inducibility of authentic YZE mRNA in visceral endoderm: for example, YZE.1 accumulated the highest levels of YZE mRNA, but showed little readthrough transcription. Non-inducers such as YZE.2 and YZE.4 showed markedly different levels of this readthrough transcription. Thus it is not clear what impact, if any, this phenomenon had on the experiments.

Nevertheless, it is important to point out that the activation of the YZE locus during differentiation did not require a derepression of a transcriptionally inactive region, events which have been hypothesized to require significant changes in chromatin structure⁴³. Whether such events occur during the activation of the AFP gene and whether the exogenous genes have bypassed such a requirement are unknown.

For five of the YZE transformants the regulation of YZE mRNA expression during differentiation was qualitatively identical to authentic AFP mRNA in that it was induced selectively in visceral endoderm. No transformants were isolated in which the expression of YZE mRNA was either constitutive or detected in parietal endoderm. Thus we must conclude that the DNA in YZE is sufficient to confer tissue specificity.

The major difference between the activation of the endogenous AFP and exogenous YZE mRNAs was the relatively low levels of YZE mRNA. Both mRNAs were detected by day 7 of aggregation, but after day 7, the levels of AFP mRNA rose dramatically while YZE mRNA levels increased only 2–3-fold. It has been argued that there are two stages in the activation of AFP gene expression; an initial activation event that occurs during the organization of the visceral endoderm and a secondary induction that occurs after formation of the basement membrane^{24,25}. If this is the case, the YZE minigene construct contains the element(s) required for the initial tissue-specific activation but not for the subsequent induction.

The mechanism underlying the second increase in AFP mRNA after day 7 has not been elucidated—it may occur via a selective stabilization of AFP mRNA, which is known to have a very long half life (60–120 h) in visceral yolk sac⁹. In that case, the failure of YZE mRNA to accumulate could be due to

the absence of internal sequences necessary for stability. On the other hand, the second increase in AFP mRNA could occur via an increase in transcription rate, in which case the limited induction of YZE mRNA could be explained by an inefficient transcriptional induction. The latter could be the result of position effects due to random integration and/or the absence of regulatory sequences in the minigene.

There is an interesting correlation between the number of integrated copies of YZE DNA and the inducibility of YZE mRNA. Five of the six multiple-copy transformants induce YZE mRNA in visceral endoderm whereas none of the six low copy number transformants is positive for YZE mRNA after differentiation. There are at least three possible explanations for this correlation. First, it may be a dosage effect. In order to detect YZE mRNA there must be multiple copies of the template to compensate for very inefficient expression of the exogenous template. However, there does not seem to be a firm correlation with copy number because the transformant that accumulates the highest level of YZE mRNA, YZE.1, contains the least number of YZE DNA copies among the multiple-copy transformants. Second, as has been frequently observed in other systems, a large proportion of the YZE DNA in the multiple-copy transformants appears to be integrated in tandem arrays as judged by digestion of genomic DNA with a restriction enzyme that cleaves the input DNA at a single site (data not shown). The tandem arrays may serve to isolate the internal copies of the YZE templates from any repressing activity from the surrounding chromosomal DNA. A third explanation may be related to the fact that none of the low-copy transformants has integrated an intact copy of the 14 kb of 5' flanking YZE DNA whereas at least several intact copies are present in each multiple-copy transformant (data not shown; see Fig. 1). Therefore, the possibility remains that an essential element in the 5' flanking DNA required for activation has been deleted in all the low-copy transformants. It will be important to generate full-length single-copy transformants to distinguish between these models.

In conclusion, we have introduced into F9 cells an internally deleted AFP gene which contains sufficient DNA information to confer tissue-specific expression. The low expression of this transfected gene suggests that additional elements, related either to sequences not contained within the minigene or to the sites of integration of the DNA, may have a role in the extent of inducibility of the AFP gene.

We thank Beverly Hazel for technical assistance and A. Minty and J. Seidman for α -actin and H-2 cDNA clones, respectively. This work was supported by NIH grants CA06927, CA28050 and RR05539 and by an appropriation from the Commonwealth of Pennsylvania. R.W.S. was supported by a USPHS postdoctoral fellowship.

Received 29 March; accepted 24 May 1984.

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A radio lobe over the galactic centre

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Extended radio continuum emission from the central region of our Galaxy has been extensively studied in relation to diffuse H II regions near the galactic plane¹ using radio survey data²⁻⁴. However, since Kerr and Sinclair⁵ called attention to a 'jet'-like feature in the central region, no extensive research has been done on off-plane diffuse radio emission, which is characterized by radio spurs at steep angles to the galactic plane. We report here a prominent off-plane radio lobe structure above the galactic nucleus, which has been revealed during a 10-GHz continuum survey of the galactic plane.

The observations were made in April 1983 using the 45-m telescope at the Nobeyama Radio Observatory (NRO)⁶. The half power beam width (HPBW) was 2.6 arc min at the central frequency of 10.5 GHz. The first side lobe level was below -20 dB, which provided high dynamic range measurements. We used a cooled parametric amplifier combined with a Dicke switching system. The bandwidth was 500 MHz and the system temperature was 150 K. We mapped a squared area of $4^\circ \times 4^\circ$ centred on the galactic centre by scanning in the direction of the latitude at an interval of 1.2 arc min. The integration time per beam area was effectively 1.4 s. The two extreme edges of each scan at $b = \pm 2^\circ$ were taken as the zero levels. The data were reduced using the radio astronomical reduction system at the NRO.

The 10-GHz continuum maps reveal spur structures emerging from the galactic plane towards high latitudes⁶. The most prominent are the two at $l = 0.2^\circ$ and at $l = -0.6^\circ$, both extending towards positive galactic latitudes at steep angles to the galactic plane. They already appear in the previous surveys at lower frequencies²⁻⁴.

We emphasize here that both the spurs bend convex with respect to the galactic centre and are connected to a radio arc at around $b = 1.2^\circ$ to form an Ω shape. Figure 1 shows the whole structure, where the map has been smoothed to a 3.6 arc min HPBW. Figure 2 shows a grey-scale representation of the same region at a 3 arc min resolution, where a background smooth component of scale sizes $> 1^\circ$ has been subtracted using a background filtering (BGF) method⁷, which enhances finer-scale structures. Figures 1 and 2 demonstrate that the two spurs form a giant Ω -shaped radio loop—the GCL (galactic centre lobe). The geometrical centre of the lobe is at $(l, b) = (-0.3^\circ, 0.6^\circ)$. Its angular diameter in the longitude direction is about 1.06° , which corresponds to a linear size of 185 pc at a 10 kpc distance. The height of the lobe is about 1.2° from the galactic plane, or about 210 pc, if the lobe is located at the galactic centre region.

We emphasize that the lobe has steeper gradients of radio emission towards the outer edges. This is seen in Fig. 3 which shows a cross-section of the lobe along a line of constant latitude at $b = 0.4^\circ$. Figure 3 also shows a cross-section along the same line at 5 GHz taken from the Bonn survey³. The cross-sections show a brightness distribution typical of a compressed shell. This may indicate that the lobe is a result of an explosive or an energetic outflow phenomenon. The cross-sections are well represented by a superposition of two components: a lobe component with two sharp maxima and a smooth background component as indicated by the dashed lines.

The background component has a steep spectral index $\alpha = -0.6$ to -1 (surface brightness $\propto \nu^\alpha$), which indicates a nonthermal origin. On the other hand, the lobe component, after subtraction of the background component, has a flat spectrum of $\alpha = -0.1$. Such a flat spectrum is not expected for a shell type

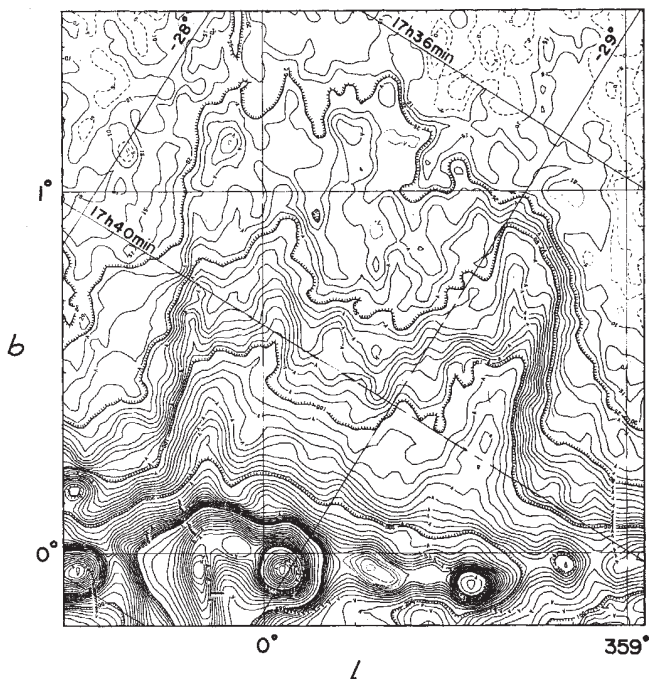


Fig. 1 A 10.5-GHz radio continuum map of the galactic centre region showing the whole structure of the GCL. The map has been convolved to a 3.6 arc min HPBW gaussian beam. The numbers on contours are in unit of 5.77 mJy per beam of 2.6 arc min HPBW ($= 9.1 \times 10^{-23} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1} = 2.7 \text{ mK } T_b$).

supernova remnant (SNR), and we may exclude the possibility that the GCL is a nearby foreground SNR. The total flux density of the lobe component integrated at $b \geq 0.2^\circ$ is calculated to be 132 ± 10 Jy. The flat spectrum suggests a thermal (free-free) radiation from an ionized gas. If the lobe is composed of ionized H II gas, we can estimate its electron density and mass. If we assume a temperature of 5,000 K, which has been derived for extended low-density H II disk¹, the total mass of the ionized gas in the lobe is estimated to be $4 \times 10^5 M_\odot$. The mean electron density in the lobe is $n_e \approx 5 \text{ cm}^{-3}$; the total thermal energy is $\sim 3 \times 10^{50}$ erg. If the gas is ionized by radiation, the number of UV photons required is $\sim 10^{51} \text{ s}^{-1}$, about one-third of that required for ionization of the extended low-density H II disk around the nucleus¹.

The geometrical appearance of the GCL in wider area maps of the galactic centre region²⁻⁶ strongly supports its association with the nuclear disk. We emphasize that the galactic plane at $10^\circ \geq l \geq -10^\circ$ is a region where strong radio sources are rarely found except for the nuclear region at $2^\circ > l > -2^\circ$. The flat spectrum of the GCL suggests a common origin for the lobe gas and the extended low-density H II gas in the disk. To examine the association of high-velocity gases with the GCL, a survey of the CO line emission has been carried out using a 4-m millimetre-wave telescope at Nagoya University. Preliminary CO line data (I. Suwa, Y. Fukui, Y.S. and T.H., in preparation) show a possible association of a high-velocity ($v_{\text{LSR}} \sim 100 \text{ km s}^{-1}$) feature with the western ridge. Such a high velocity cannot be expected from the foreground and background gases. From these arguments we conclude that the GCL is probably associated with the nuclear disk.

The southern extension of the GCL remains uncertain. However, Fig. 1 and a larger area map at 10 GHz (see ref. 6) show a possible connection of the eastern ridge with the 'radio arc' at $l = 0.2^\circ$, $b = 0^\circ$. In the larger area map, we can also find a possible extension of the ridge towards the south reaching to $(l, b) = (0.1^\circ, -0.8^\circ)$, where the ridge is rather broad. We have

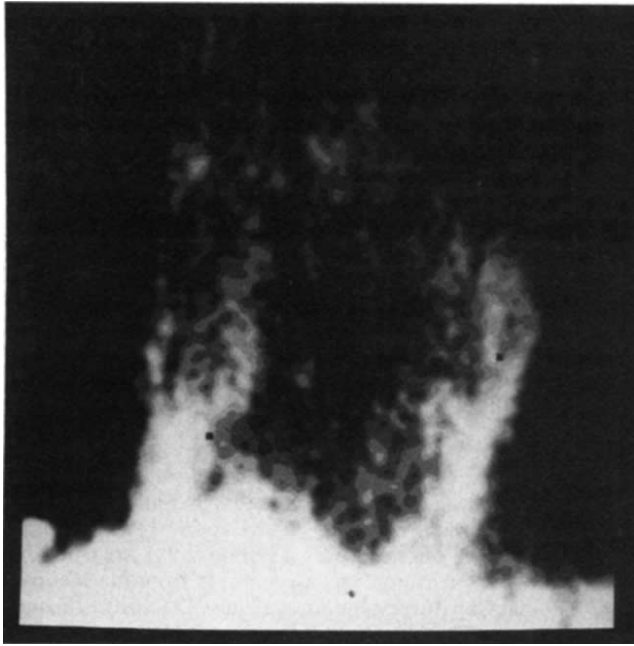


Fig. 2 The same region as in Fig. 1 but on a grey scale. The background smooth structure with scale sizes $> 1^\circ$ has been subtracted from the original, so that the ridge structures are clearer. The map has been convoluted to a 3 arc min beam.

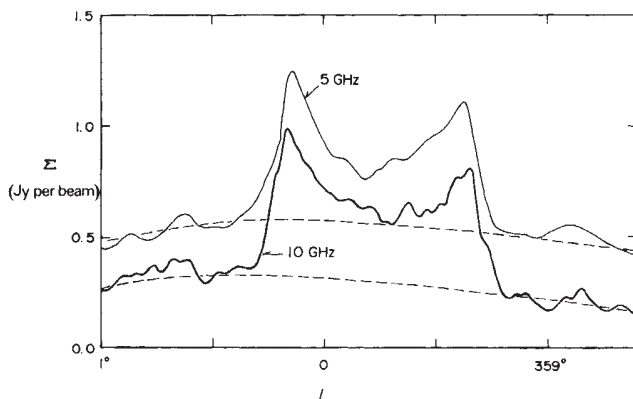


Fig. 3 Brightness distributions across the GCL at 5 and 10 GHz both in Jy per 2.6 arc min beam along a line at $b = 0.4^\circ$, showing that the lobe has a cross-section typical of a shock-compressed shell. The background component, as indicated with the dashed lines, has a steep spectrum of $\alpha \approx -0.6$ to ~ -1 , while the lobe component has a flat spectrum of $\alpha \approx -0.1$.

no clear southern extension associated with the western ridge, although we can see a small spur-like feature starting from a strong peak at $(l, b) = (-0.6^\circ, -0.1^\circ)$ and extending to $(-0.5^\circ, -0.3^\circ)$. To see the situation more clearly, however, we need higher resolution observations of the region close to the galactic plane.

We propose the following possible formation mechanisms for the origin of the GCL.

(1) Channelled exhaust from the nuclear disk: a shock wave produced by an explosion or an energetic outflow of matter from the nuclear region will be channelled in the direction perpendicular to the galactic disk⁹. If the distribution of gas in the disk is anisotropic, the flow will be channelled in a one-sided cone and blown off into a less-dense side, resulting in an Ω -shaped lobe. To blow off the gas of mass $4 \times 10^5 M_\odot$ to the height of 100–200 pc of the galactic plane requires the explosion energy to be $\geq 10^{51}$ erg. If the expansion velocity is comparable to that of the high-velocity CO gas, the explosion energy will be of the order of 10^{54} erg, far larger than that of a single

supernova. A channelled exhaust can also be produced by a magnetic field running perpendicularly to the disk and to the existence of an accreting gas disk⁹: the accreting gas twists the magnetic lines of force, and the twist propagates along the field, with which the ionized gas is accelerated to produce a cylindrical outflow perpendicular to the disk plane, reproducing the GCL shape (Y. Uchida and K. Shibata, in preparation).

(2) Galactic loop prominence: inflation of a giant loop of magnetic tube filled with ionized gas, similar to a solar loop prominence, may produce an Ω -shaped lobe in the halo. The inflation may be triggered by the Parker-type instability in a magnetized nuclear disk in the presence of cosmic rays. In this case, the magnetic energy should be comparable with, or greater than the thermal and gravitational energy of the gas involved in the lobe. This leads to a field of $\sim 20 \mu\text{G}$.

Off-plane radio structures perpendicular to galaxy disks have been observed in the central regions of several edge-on spiral galaxies^{10,11}. In particular, the radio lobe found in the central region of NGC3079 suggests inflation of magnetic bubbles from the nuclear disk into the halo¹¹. The Ω -shaped lobe in our Galaxy may be a similar phenomenon, although on a different scale: the linear size of the GCL is ~ 200 pc, while those in the other spiral galaxies are ~ 1 –3 kpc. Such a channelled exhaust perpendicular to the galaxy disk or a 'cosmic jet', of various scales, is likely to be a common phenomenon associated with active nuclei of spiral galaxies including our own Galaxy.

Received 2 March; accepted 24 May 1984.

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Search for pulsed optical emission from the millisecond pulsar PSR1937+214

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A search has been made for optical pulses from the 1.6-ms pulsar PSR1937+214, using the 3.9-m Anglo-Australian Telescope (AAT). Despite previously reported indications¹ of pulsed emission at this period from the red star discussed by Djorgovski², we now believe the results of the search to be negative with an upper limit on the R magnitude of the pulsed emission of ~ 25 . Furthermore, astrometric observations show that the red star is displaced about 2.4 arc s north of the pulsar position and, hence, that it is likely to be a chance association.

Optical pulses have previously been detected from the Crab pulsar³ and the Vela pulsar⁴, both of which have relatively short periods, 33 and 89 ms respectively. The relative luminosity of the optical emission from these pulsars is approximately in accord with the relation proposed by Pacini⁵, $L \propto B_0^2 P^{-10}$, where L is the optical luminosity, B_0 is the surface magnetic flux density (a dipole field structure is assumed) and P is the pulsar period.

Table 1 Star positions from the AAT-CCD image (FK4 system)

	Position (1950)	
	RA	Dec
Reference positions from Palomar plate		
Star A	19 h 37 m 29.69 s	+21° 27' 16.3"
Djorgovski's star	19 37 28.78	+21 28 03.3
Reference positions from UKSTU plate		
Star A	19 37 29.69	+21 27 16.9
Djorgovski's star	19 37 28.80	+21 28 03.7
Pulsar VLA position	19 37 28.72	+21 28 01.3
Pulsar timing position	19 37 28.722	+21 28 01.45

The millisecond pulsar⁶, PSR1937+214, has the very short period of 1.558 ms, a factor of 20 less than that of the Crab pulsar. This, together with the strong period dependence in Pacini's relation, immediately suggested this pulsar as a strong candidate for optical pulse searches. On the basis of approximate positional coincidence, Djorgovski² suggested as a candidate for the pulsar a red star of approximately 20th magnitude. Enthusiasm for searches for optical pulses was tempered by the observation^{7,8} that the pulsar has a very small period derivative, $dP/dt \approx 10^{-19}$, and hence relatively small magnetic field, $B_0 \approx 5 \times 10^8$ G. From Pacini's relation, the ratio of optical luminosities for the millisecond and Vela pulsars is $L_{1937}/L_{\text{Vela}} \approx 75$.

However, the millisecond pulsar is more distant than the Vela pulsar, with estimates varying from 2.5 kpc, based on the dispersion measure⁶, to 5 kpc, based on neutral hydrogen absorption observations⁹. For the smaller distance, PSR1937+214 and the Vela pulsar might be of comparable apparent brightness; however, if the larger distance is correct then, including the effects of interstellar extinction, PSR1937+214 would be about six magnitudes fainter.

Time-resolved observations were made using the 3.9-m AAT on six nights in 1983, 20–21 April, 15 May and 3–4 August. For the April and August observations a photometer system having a GaAs photocathode was used directly at the $f/15$ Cassegrain focus. On 15 May a similar photometer system was used at the $f/8$ auxiliary Cassegrain focus. Most observations were made with a red (OG590) filter; a B filter was used for one observation on 20 April and for one observation on 4 August there was no filter. The aperture size was 3.5 arc s for all observations. The data recording system was that described by Jones *et al.*¹⁰. A specially built 'photon event timer' accepts 1 Hz and 1 MHz reference signals from the observatory clock and pulses from the photometer. The clock signals control a counter which is strobed out to a Camac FIFO module whenever a photon event is detected. The instrument control computer reads the photon arrival times from the FIFO and writes them on to magnetic tape in a compressed form, along with other timing information which defines the absolute UTC of each detection to 1- μ s resolution.

For the April and May observations the observatory clock

was aligned to UTC using a synchronization system based on television transmissions. Unfortunately it was subsequently discovered that there were problems with the synchronization system, and so UTC times for these sessions are uncertain. The clock stability was sufficient to integrate each observation but the epoch was sufficiently uncertain to prevent synchronization of observations made on different nights. For the August observations, the observatory clock was replaced by a Rb standard aligned to UTC to better than 5 μ s.

In both on-line and off-line programs, the data were binned synchronously with the pulsar period using a barycentric pulsar ephemeris derived from radio timing observations (A. G. Lyne and J. H. Taylor, personal communications). Photon arrival rates were also converted into an analogue signal which was monitored on a chart recorder and also supplied to the telescope control computer for use in an automatic aperture centering program.

The telescope was positioned by centering the aperture on star A in Djorgovski's nomenclature and offsetting to the desired position. An autoguider was then used to maintain the telescope tracking. The initial centering of the aperture was accurate to 0.2 arc s and the offsetting was accurate to 0.1 arc s.

Astrometric measurements were made from glass copies of the Palomar Sky Survey E plates, a short (30-min) exposure on a IIIaF plate (RG630 filter) from the UK Schmidt Telescope Unit (UKSTU) which was centred on the field, and a Charge Coupled Device (CCD) image of the field taken by I. M. McHardy and B. A. Cooke on 15 April using the AAT. The CCD image, shown in Fig. 1, was taken with a red filter, giving it an effective spectral response similar to that of the GaAs photometer system. The primary reference frame was determined from measurements of 10 SAO stars on the Palomar and UKSTU plates and the secondary reference frame on the CCD image from measurements of 21 stars. Positions obtained for star A and the candidate star are given in Table 1. We note that our positions for the secondary reference stars agree well with those of Condon (see ref. 2), with the exception of star D, which apparently has a large proper motion. We believe that errors in the positions based on the UKSTU plate are ≤ 0.5 arc s and are probably dominated by uncertainties in the primary reference frame. Substitution of AGK3 positions for the primary reference stars made no significant difference to the derived positions. For comparison, radio positions of the pulsar from Very Large Array (VLA)⁶ and timing (J. H. Taylor, in preparation) measurements are also given in Table 1.

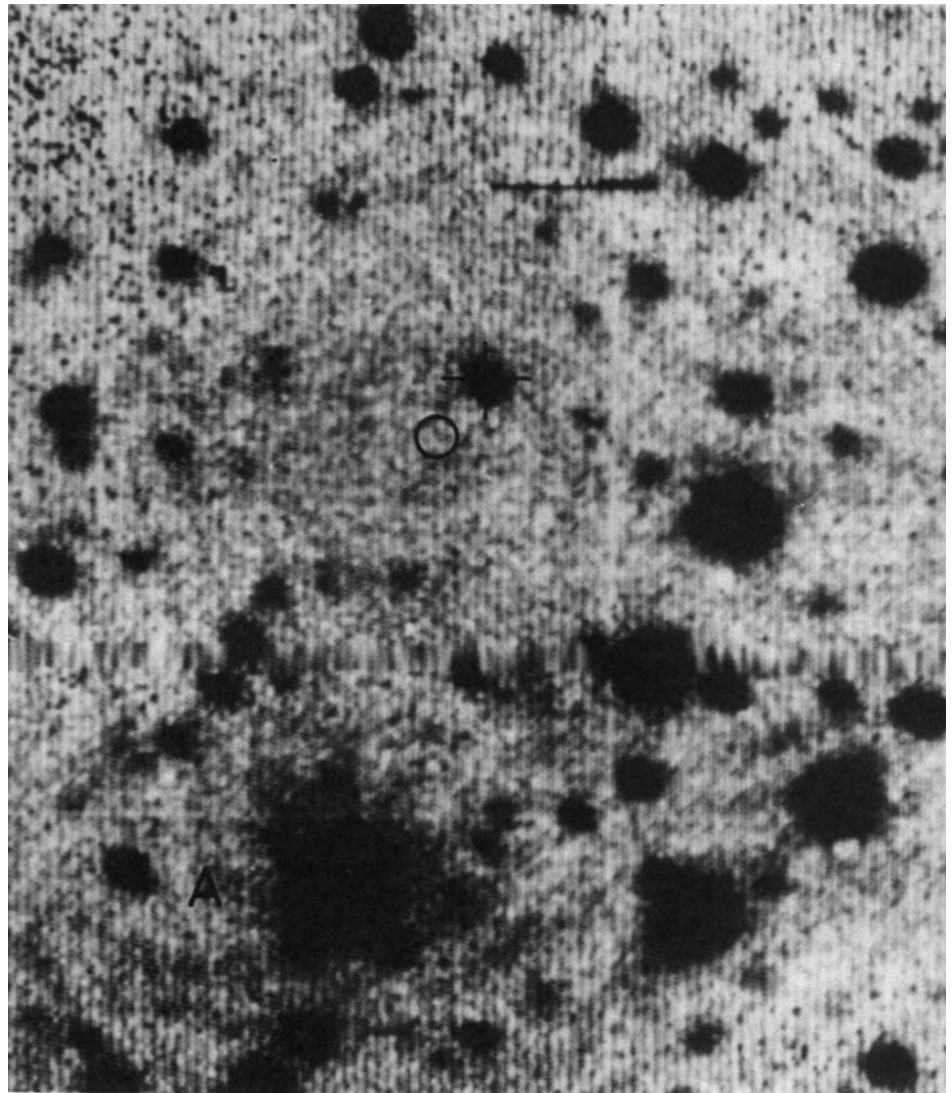
Djorgovski's star appears brighter relative to other stars on the CCD image than it does on the Palomar E plate. However, an 85-min exposure on a IIIaF plate through an RG630 filter taken by the UKSTU on 26 September 1983 shows no evidence for any change and hence suggests that the apparent difference is due to the differing spectral responses of the CCD camera and the photographic plates. Observations on 20 April gave a photometer count rate of 110 ± 3 Hz for this star, which corresponds to an R magnitude of 18.90 ± 0.03 after correction for 0.03 mag of extinction.

The derived position for Djorgovski's candidate star is 0.08 s east and 2.4 arc s north of the VLA radio position⁶. It disagrees

Table 2 Observations and magnitude limits for pulsed emission from PSR1937+214

Date 1983	Position	Filter	Seeing (arc s)	Observing time (min)	Magnitude limit
20 April	Djorgovski's star	Red	3.5	31	24.9
20 April	VLA position	Blue	4.0	36	25.2
21 April	Djorgovski's star	Red	4.0	77	25.4
15 May	Djorgovski's star	Red	2.5	54	24.7
15 May	VLA position	Red	2.5	32	24.5
3 August	Djorgovski's star	Red	3.5	103	25.0
3 August	Timing position	Red	3.5	79	25.0
4 August	Timing position	None	3.5	149	26.3
4 August	Timing position	Red	3.0	65	25.2

Fig. 1 Red-sensitive CCD image of the PSR1937+214 field from AAT observations on 15 April, 1983. North is up, east is to the left and the region shown is about 85×98 arc s. The astrometric reference star A is indicated, the cross marks Djorgovski's candidate star, and the circle indicates both the aperture size and the position used as reference when determining the magnitude of the candidate star.



with the position offsets given by Djorgovski², which place the candidate star 0.11 s east and 1.5 arc s south of the VLA position. An independent measurement of the radio position has been obtained from pulse timing observations; after allowance for the E-terms of aberration, the timing position is within 0.2 arc s of the VLA position. It seems very unlikely that Djorgovski's star is associated with the pulsar. It is probably a red giant¹¹. There is no star to the CCD image limit at the radio position.

The observations and results are summarized in Table 2. Limits on the magnitude of the pulsed emission are derived assuming a pulse duty cycle of 0.125. For the April observations the magnitude scales are based on observations of star no. 8 in the sequence of Graham¹² and for the May observations on star E7/S of Graham¹³. For the August observations star A was used as a secondary reference. The *R* magnitude of this star, determined on 15 May, is 14.4. The no-filter observation of 4 August was calibrated by taking the *V* magnitude of star E7/S (ref. 13) and correcting for the poorer seeing at the object zenith distance.

There is no evidence for pulsations at the predicted pulsar period in the data from the August observations. As the timing system was reliable and observing conditions were reasonable on those nights, we conclude that the pulses observed in the earlier sessions were spurious and that there is no evidence for optical pulsed emission from either Djorgovski's star or the pulsar position. The coincidence of pulse features on 20 and 21 April which led to our earlier conclusion of significance¹ was based on incorrect clock data and disappeared when corrections for clock errors were made, although there still remains some

doubt about the reality of these corrections. Scans in period about the nominal value show that the pulse features observed in data recorded on 20, 21 April and 15 May individually are of low significance.

The best limits given in Table 2 for the magnitude of the pulsed emission correspond to a detected photon rate of ~ 0.2 Hz. If we assume an overall detection efficiency of 5%, a distance to the pulsar of 5 kpc (ref. 9), interstellar absorption of 1.5 mag kpc⁻¹, and that the pulsar beams into a cone of width 45° (corresponding to the 0.125 duty cycle assumed above), then the limit on the luminosity of the pulsar is $\sim 10^{32}$ erg s⁻¹. With the same beaming assumptions, a distance of 0.5 kpc, no absorption, and a detected photon rate of 2 Hz (ref. 14), we find that the luminosity of the Vela pulsar is about 10^{28} erg s⁻¹. Pacini and Salvati¹⁵ predict a luminosity of $\sim 10^{30}$ erg s⁻¹ for PSR1937+214, that is about two orders of magnitude below our upper limit. However, their value for the observed luminosity of the Vela pulsar is about an order of magnitude larger than the value given above, presumably because of different assumptions about the beaming. This would suggest that our upper limit is even further above their prediction. If the pulsar is as distant as 5 kpc, it is impossible to reach the predicted level, at least from ground-based observations.

We thank I. M. McHardy and B. A. Cooke for the CCD image of the PSR1937+214 field, the UKSTU for taking the two plates of the field, A. G. Lyne and J. H. Taylor for unpublished timing data for PSR1937+214, and I. K. Harvey, J. S. Thorn and P. R. Dencher of the CSIRO Division of Applied Physics for

providing the Rb time standard for our August observations. We also thank the director of the AAT for allocating observing time on 15 May and D. L. Jauncey and his colleagues for trading observing time with us in August. Software produced by the SERC Starlink project was used to reduce the CCD observations.

Received 14 May; accepted 21 June 1984.

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Relativistic deflection of radio signals in the solar gravitational field measured with VLBI

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For an observer on Earth the general theory of relativity (GR) predicts an apparent outward displacement of a star seen at the Sun's limb of 1.75 arc s. A generalized formulation for gravitational deflection of light¹ includes a parameter γ which ranges from 1 (GR) to -1 (no deflection). Early total eclipse measurements² constrained γ by 20–40%, permitting alternative gravitational theories (for example, ref. 3). Radio interferometer measurements of deflections of extragalactic objects^{4,5} and timing measurements of spacecraft signals⁶ have all been consistent with GR with increasing accuracy. We now report the most sensitive measurement of deflection so far achieved, in which very long baseline interferometry (VLBI) observations yield a value of γ of 1.008 with a 1σ formal standard error of ± 0.005 .

The observations reported here were collected under the aegis of projects MERIT⁷ (Monitor Earth Rotation and Intercompare the Techniques of observation and analysis), POLARIS^{8,9} (POLar-motion Analysis by Radio Interferometric Surveying), and IRIS¹⁰ (International Radio Interferometric Surveying). All of these projects involve the application of VLBI to geodesy, and specifically to the monitoring of polar motion and Earth rotation, and to the development of a global geodetic network to study plate tectonics. The observing schedules were not planned to optimize determination of γ . In fact, it was fortuitous that three of the radio sources used in these programmes happened to lie near the ecliptic, and therefore had very small Sun angles during certain times of the year. It was also fortunate that the observations were all dual frequency (X and S band), enabling us to remove the effects of the solar corona as well as the Earth's ionosphere, that the observations were well distributed in right ascension and declination and that very nearly the same sources were observed throughout the 40-month span of the data. We were, therefore, able to obtain an excellent estimate of γ from a set of observations that was collected for a quite different purpose.

The data set consists of 41,236 delay and delay-rate observations collected during 163 observing sessions conducted between September 1980 and January 1984. Twelve observatories in the United States and Europe participated in the programme,

although most observations used only the 3,100-km interferometer formed by the Westford Observatory in Massachusetts and the G.R. Agassiz Station (GRAS) in Texas. All of the observatories have the Mark III VLBI system¹¹ of instruments. The observing sessions were typically 24 h in duration, with each interferometer collecting 100–200 delay observations per session. The r.m.s. scatter of the delay residuals about the mean for each session is typically 100 ps. About 14 radio sources were observed in each individual session, and the full data set includes observations of 20 sources. Of these, two (3C273B and 0106+013) are about 5° away from the ecliptic, and one (0J287) is about 2.6° away from the ecliptic. A fourth source (0235+164) is only about 1° from the ecliptic, but this source proved to be too weak to produce reliable fringes and was dropped from the observing schedules without ever having been observed close to the Sun.

The distribution of the observations as a function of Sun angle is shown in Fig. 1, with the Sun angles divided into 1° bins. Out of the 41,236 delay observations, 184 were made at Sun angles <10° and 26 were made at Sun angles of <5°. The observations closest to the Sun were made in early August 1982 and 1983, when 12 observations of 0J287 were made at ~3° from the Sun. The magnitude of the gravitational deflection for these points is of the order of 150 m arc s.

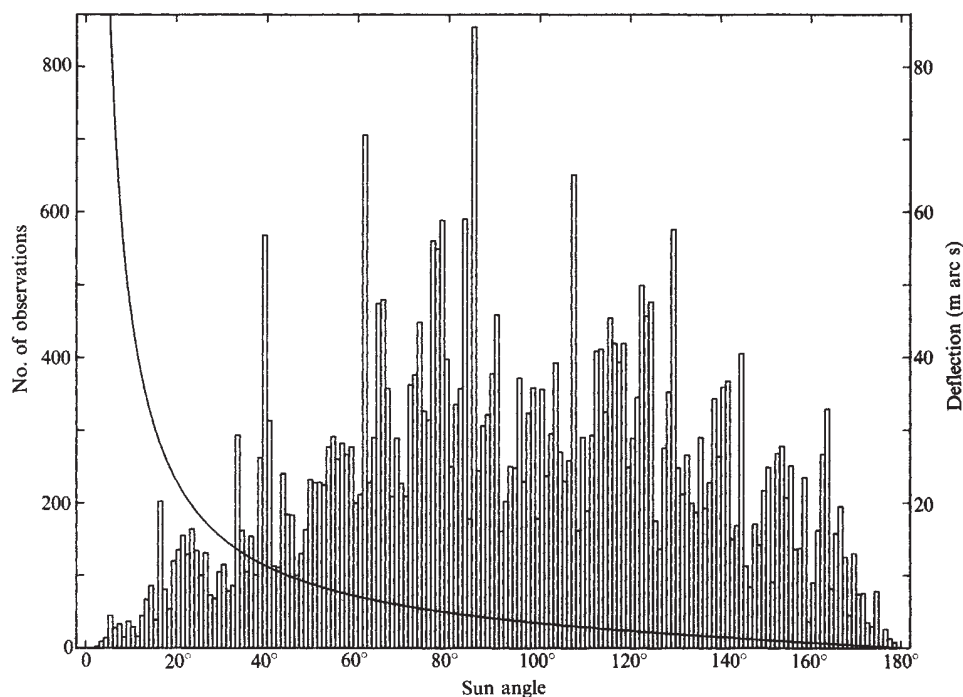
The curve in Fig. 1 shows the magnitude of the gravitational deflection as a function of Sun angle with γ set to 1. The deflection changes rapidly near the Sun, but then levels out and is still larger than 4 ms as far as 90° from the Sun. The deflection does not drop to 0.2 m arc s, which is the level of the 1σ formal errors for the source coordinates estimated from combined solution, until the Sun angle reaches 175°. In other words, the precision of the VLBI observations is so high that the deflections caused by the solar gravitational field are significant virtually everywhere in the sky. The apparent displacements of the sources by the solar gravitational field thus have periodic signatures, with 1 yr periods. These periodic signatures decrease the correlation between the parameter γ and other parameters in the solution, especially the source coordinates.

The correlations between γ and the other parameters in our solution are remarkably small. The largest correlation is between γ and the right ascension of the source 2134+00, with a coefficient of only 0.11. Several other source right ascensions were correlated with γ at about this same level. The largest correlation with a station coordinate involved the x coordinate at GRAS, and had a value of 0.025. The correlations between γ and the clock and atmosphere parameters were also quite small. The coefficient values ranged from ~0.05 on days in which a source was close to the Sun, to about 0.005 on days in which no source was close to the Sun. These extremely small values of correlation coefficients imply that the usual sources of systematic errors affecting VLBI observations (such as atmosphere and clock effects and station and source coordinate errors) will not significantly contaminate our estimates of γ .

Neutral atmosphere effects on the observed delays have been removed by a two-stage process. First, a model based on surface measurements of temperature, pressure and humidity was used in the fitting process. Second, the VLBI observations themselves were used to estimate a small additive correction term to the model. The remaining atmospheric errors are believed to be at the level of <1 m arc s. Despite their small correlations, these errors may very well be the dominant source of systematic error in our estimate of γ .

Another possible source of systematic error involves the effect of possible errors in the nutation model used in reducing the observations. Our reductions used the IAU 1984 standard nutation model, based on the work of Wahr¹². Errors in this series, especially errors with a period close to 1 yr, could perturb the source coordinates in a manner which correlates with the effects of gravitational deflection. Preliminary estimates of the correlations between γ and daily nutation angle parameters indicate that the magnitude of these correlations will be similar to that of the clock and atmosphere parameter correlations.

Fig. 1 Histogram showing the Sun angle distribution of the VLBI observations. The observations have been collected into 1° bins for plotting. The smooth curve shows the expected deflection of the source position in m arc s, calculated for $\gamma = 1$. The deflection is large enough to be detected as far as 175° from the Sun.



The refraction caused by the solar corona at 3° from the Sun is $\sim 2\text{--}7$ ms at X-band, depending on the level of solar activity⁴. The larger value is $\sim 5\%$ of the gravitational deflection. The percentage decreases rapidly at larger Sun angles because the coronal refraction decreases approximately as the square of the Sun angle while the gravitational deflection decreases nearly linearly. Like the Earth's ionosphere, the coronal refraction is dispersive, varying inversely with the square of the observing frequency. The technique of making simultaneous observations at widely separated frequencies (X and S band), implemented primarily to address the ionospheric refraction, served equally well to remove the coronal refraction effects, obviating the use of corona models with their attendant systematic errors.

The observed data were combined in a least-squares adjustment to determine γ . In addition to γ , the parameters in the solution included all of the source coordinates (except the single source right ascension which served to determine the origin of right ascension), baseline coordinates, polar motion and Earth rotation parameters, clock error coefficients and atmosphere zenith delay parameters. The value of γ which resulted from this solution was 1.008 ± 0.005 . The quoted uncertainty is the 1σ formal standard error based on observation weights which have been adjusted so that the r.m.s. value of the weighted residuals is close to unity. Although this value of γ is slightly more than 1σ larger than the value predicted by the general theory of relativity, we do not believe that the difference is large enough to constitute a serious discrepancy from that prediction. The remaining systematic errors in the solution could be at least as large as the expected statistical errors.

To test the sensitivity of this determination to changes in the set of parameters used in the least-squares solution, we ran solutions with the source coordinates and station coordinates fixed, and solutions with the Earth tide Love numbers adjusted. None of these changes affected the formal error of the determination of γ by more than 0.0001. The only change which affected the value of γ by more than 1σ was fixing the source coordinates at their *a priori* values, some of which were as much as 15 formal standard errors away from their adjusted values. This change caused the estimated value of γ to increase by about twice its formal standard error. The determination of γ , therefore, seems to be fairly stable against reasonable changes in the selection of the set of parameters to be adjusted, although our set of *a priori* source coordinates apparently needs some refinement.

To test the sensitivity of the solution to the distribution of observations in Sun angle, we ran a solution which included only those days containing a source within 10° of the Sun. This solution included only 23 observing sessions, with 6,570 of the 41,236 observations. The resulting value of γ was 1.016 ± 0.008 , about one formal standard error away from our combined solution result. Thus, although we can come close to our combined result with only 15% of the data, the remaining 85% can be seen to contribute substantially to the determination of γ .

This is the most sensitive measurement yet made of the deflection of electromagnetic radiation by the solar gravitational field. Although the precision of the determination of γ is not as good as that obtained from the spacecraft tracking time delay measurements, it is important because it is totally independent of that result, and has few, if any, common sources of systematic error. An even more important feature of this determination is that it was obtained as a by-product of a continuing observation programme. The measurements will continue to amass through the foreseeable future, and thus the precision of the determination of γ will continue to improve slowly. In addition, substantial improvements are expected in the measurements in the near future: for example, two new observatories located in Florida and the FRG have just begun to participate regularly in the observations. The station in the FRG is particularly important because it greatly increases the number of observations obtained with long (6,000 km) USA–Europe interferometers. Also, substantial improvements are expected in the signal-to-noise ratios of the raw observables as the stations become equipped with more sensitive receivers later this year. The increased sensitivity will allow us to obtain reliable data at even smaller Sun angles than is presently possible. Also, as we noted above, no effort has been made thus far to optimize the collection of data at small Sun angles. It will be possible to do so in the future, for example, by increasing the duration of the observing scans close to the Sun, without significantly perturbing the primary objectives of the geodetic programmes. In particular, we should be able to obtain a great deal of coverage on very long baselines for the conjunction of 0J287 with the Sun in August 1984. In addition, the source 0229 + 131 is only $\sim 1.5^\circ$ from the ecliptic plane and has recently been shown by a NASA survey experiment to be very strong even when observed with transcontinental baselines (D. B. Shaffer, personal communication). It will be added to our observing programme in time for its conjunction

with the Sun in May 1984. It should, therefore, be possible to improve this determination of γ in the future at virtually no cost.

Received 17 April; accepted 12 June 1984.

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Continental distribution as a forcing factor for global-scale temperature

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Since the advent of the continental drift hypothesis, changing continental geometries have been proposed as an explanation for long-term temperature variability^{1–9}. The climatic influence of a few specific past geographies has been investigated quantitatively^{6,10–12}, but these studies do not indicate the potential temperature variability due to continental positions. This problem has been examined only with simple climate models having limiting assumptions such as no cloud cover⁵. Here idealized continental geometries are used as boundary conditions in a simulation using a general circulation model (GCM) of the atmosphere. The range in model simulated globally-averaged surface temperature is 7.4 K with a difference in polar surface temperature of up to 34 K. The simulations suggest a substantial climatic sensitivity to continental positions with the coldest global climate when land masses are in high latitudes. Although the simulations have not captured theoretical limits of climatic variability due to continental positions, present-day geography is near the cold end of this spectrum.

Two idealized continental geometries based on present-day total land area, a tropical land belt 17° N to 17° S and polar land 'caps' from 90° to 45° N and S, are used as extreme limits of continental configurations in the community climate model (CCM), a spectral GCM at the National Center for Atmospheric Research (NCAR). In addition, the polar land case is considered with and without polar ice caps (permanent snow cover with no thickness) extending equatorward to 70° of latitude. In each case the continental elevation is specified at 750 m, the present-day average value of ice-free continents. Each experiment is then a single variable sensitivity experiment involving either the continental position of an 'average' global continent or the presence of permanent snow cover.

The characteristics of the CCM and the validity of present-day simulations compared with observations have been well documented elsewhere^{13–17}. Briefly, the model has a resolution of 4.5° in latitude and 7.5° in longitude with nine levels in the vertical. The model includes predictive cloud cover¹⁵ and a predictive hydrological cycle including evaporation, precipitation and snow cover¹⁸. The version used here is forced by specified mean annual insolation and predicts sea-surface tem-

peratures using an energy balance ocean with no ocean currents or heat capacity but which is a moisture source¹⁷. Although not a complete model of the climate system, this model is well suited for examining first-order climate problems.

Figure 1a gives the zonally-averaged surface temperatures for the model simulations with idealized continental geometries. The case with a tropical land belt has a model-simulated globally-averaged surface temperature of 295 K. Zonally-averaged polar surface temperatures are several degrees above freezing. In comparison, the polar continent simulation is cooler at almost every latitude with a maximum cooling of 12 K at the poles. The difference in globally-averaged surface temperature between these two cases is 4.6 K. The presence of a tropical land mass suppressed tropical precipitation relative to the polar land mass case, so that the differences in evaporative cooling resulted in a cooler tropical surface temperature (≈ 5 K) in the polar land case. The addition of a high albedo 'permanent snow' surface on land poleward of 70° in latitude resulted in an additional 2.8 K globally-averaged surface temperature decrease. Cooling occurred at every latitude, but predominately in the polar regions above 70° latitude. The addition of permanent polar snow accounts for $\sim 38\%$ of the total 7.4° globally-averaged surface temperature difference between the polar and equatorial idealized continent experiments.

These numerical experiments suggest that changing geography has the potential to be a substantial climatic forcing factor and one which has probably played a substantial role during the Earth's history. Note, however, that although past realistic geographies approached these extremes they were never fully realized. The record of palaeoclimates also suggests a much greater climatic variability during the past 600 Myr (ref. 19) than achieved in the idealized continental simulations. If the sensitivity of the model is appropriate then our results imply that other forcing factors in addition to continental positions have been important during Phanerozoic time. At present, it is unclear whether an explicit ocean heat transport formulation and a seasonal cycle would substantially alter the model sensitivity to continental positions.

The results described here also confirm the suggestions^{1–7} that an increased area of land at high latitudes results in colder global climates. The explanation is that land area at high latitudes represents a surface for the accumulation of high albedo snow. The role of mid-latitude continents has not been directly investigated. Previously it has been suggested that the increased area of subtropical deserts, because of their high albedos, may also have been an important climatic influence in the Tertiary global cooling trend⁶. However, recent experiments¹² suggest that increased cloud cover and evaporation over a subtropical ocean surface compensate for the higher albedo of a desert surface. Although subtropical land areas may have an important effect on atmospheric factors such as poleward transport of moisture, they have a lesser role in terms of albedo effects and should represent an intermediate case between the two extreme continental configurations investigated here.

Results for the identical model version with present-day geography and orography¹² are given in Fig. 1b in comparison with the temperature limits derived from the idealized continent simulations. Present-day geography is near the cold end of the range of the potential variability of the idealized continent simulations. The present-day climate is also near the cold end of the spectrum of palaeoclimates interpreted from the geological record. These results emphasize the important role of palaeogeography during the Earth's history. In the present-day simulation two latitude zones are cooler than the polar idealized continent case: poleward of $\approx 70^\circ$ S and between 25 and 45° N. In each case, these differences are due to topographic effects, particularly for Antarctica and Tibet.

The idealized continent simulations have not captured true climatic limits due to geographical variables. The simulations have neglected changing land-sea areas, possible effects due to multiple continents as well as topographic variability. For instance, average topography (750 m) in the polar land case could

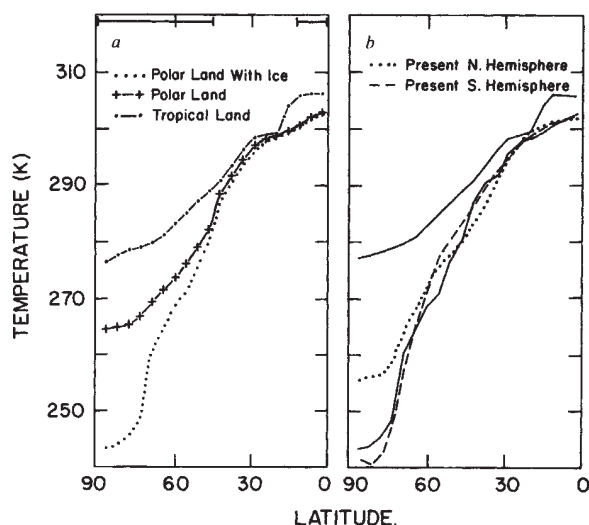


Fig. 1 *a*, Mean annual zonally-averaged surface temperatures with respect to latitude for the idealized continent simulations with all land in the tropics, all land at the poles and all land at the poles with permanent ice specified for land area greater than 70° in latitude. Bars at the top illustrate the latitudinal extent of land in the simulations. *b*, Mean annual zonally-averaged surface temperatures for a model simulation with present-day geography which compares favourably with observations, in comparison with the temperature limits (solid lines) in *a*.

enhance the areal snow cover in comparison with a more typical balance of high and low topography. Conversely, the lack of ice cap relief is likely to result in polar temperatures which are too warm. Plausibly, the interactions of the atmosphere and ocean with 'unique' distributions of multiple continents could produce substantially warmer or cooler global temperatures than the cases described here. Although the cases considered are undoubtedly extreme, there is no simple means of determining absolute theoretical limits of climate sensitivity to geographical variables. This difficulty combined with obvious model deficiencies such as the lack of seasonal variations and heat transport by ocean currents prevent us from describing maximum and minimum global temperatures achievable through possible changes in the Earth's palaeogeography. However, the results clearly illustrate the potential of continental distribution as a forcing factor for global-scale temperatures.

The National Center for Atmospheric Research is sponsored by the NSF.

Received 13 March; accepted 12 June 1984.

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Empirical approach to estimating the composition of the continental crust

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The continental crust has ultimately been extracted from the mantle and knowledge of its composition is therefore fundamental to an understanding of the chemical evolution of the Earth. Attempts to model its average chemistry are complicated by marked vertical and lateral chemical and lithological heterogeneity^{1–3} and by problems of sampling the deeper levels of the crust. We have adopted a different, empirical, approach to try and take account of geophysical and isotopic constraints by considering the major and trace element chemistry of components which are typical of various crustal levels and ages. Our estimate differs from that of the 'andesitic' model in being more siliceous and having a more fractionated rare earth element pattern. Its higher Th, U and K contents generate a higher heat production of $0.95 \mu\text{W m}^{-3}$ compared with previous estimates of 0.75 – $0.91 \mu\text{W m}^{-3}$.

Previous estimates of the major element composition of the continental crust derived by various means^{4–8} are given in Table 1. These estimates suggest that the continental crust has an intermediate chemical composition. More recent attempts at estimating the composition of the crust have centred around a model^{9,10} which considers that the dominant mechanism of crustal growth occurs through the accretion of island arcs to continents, and that the average composition of recent island arc andesites can be used to approximate the bulk composition of the continental crust. The composition so derived is given in Table 2.

We have attempted to develop a model for the composition of the continental crust which takes into account: (1) the geophysical evidence for the gross structure of the continental crust; (2) isotopic evidence for the rate of crustal growth with time; and (3) the chemistry of known components of the continental crust. We will briefly justify the basic tenets of our approach before deriving a crustal estimate.

Isotopic evidence suggests a diminishing rate of (episodic) crustal growth since the formation of the earliest continental crust ~4,000 Myr ago^{11–17}, such that by the end of the Archaean (2,500 Myr ago) as much as 75–85% of the present volume of the continental crust may have been in existence^{18–20}.

Geophysical models based on both seismic and heat flow data stress the vertical and lateral heterogeneity of the continental crust^{1,2}. Seismic data indicates that the mean composition of the bulk continental crust approximates to quartz diorite or granodiorite². Heat flow modelling^{21–23} suggests that the deeper levels of the continental crust must be severely depleted in radioactive heat-producing elements relative to the abundances observed in the upper crust. Thus, a considerable proportion of

Table 1 Estimates of the major element composition of the continental crust

	Ref. 4	Ref. 5	Ref. 6	Ref. 7	Ref. 8
SiO ₂	61.9	63.1	60.2	61.9	62.5
TiO ₂	1.1	0.8	1.0	0.8	0.7
Al ₂ O ₃	16.7	15.2	15.6	15.6	15.6
Fe ₂ O ₃	7.7	6.7	7.9	6.9	6.1
MnO	—	—	0.1	0.1	0.1
MgO	3.5	3.1	3.9	3.1	3.2
CaO	3.4	4.1	5.8	5.7	6.0
Na ₂ O	2.2	3.4	3.2	3.1	3.4
K ₂ O	4.2	3.0	2.5	2.9	2.3
P ₂ O ₅	—	—	0.2	0.3	0.2

Table 2 Compositions of crustal components and continental crust estimates

	Upper crust*	Archaean		Post-Archaean middle and lower crust§	Average continental crust	
		Middle crust†	Lower crust‡		This paper	Ref. 10
SiO ₂	66.0	66.7	61.2	59.2	63.2	58.0
TiO ₂	0.6	0.3	0.5	0.9	0.6	0.8
Al ₂ O ₃	16.0	16.0	15.6	17.2	16.1	18.0
FeO	4.5	3.2	5.3	6.1	4.9	7.5
MnO	0.08	0.04	0.08	0.12	0.08	0.14
MgO	2.3	1.4	3.4	3.4	2.8	3.5
CaO	3.5	3.2	5.6	5.9	4.7	7.5
Na ₂ O	3.8	4.9	4.4	4.0	4.2	3.5
K ₂ O	3.3	2.1	1.0	2.4	2.1	1.5
P ₂ O ₅	0.17	0.14	0.18	0.27	0.19	
Trace elements (p.p.m.)						
Cr	35	32	88	45	56	55
Ni	20	20	58	27	35	30
Rb	110	74	11	66	61	42
Sr	350	580	569	601	503	400
Ba	700	713	757	605	707	350
Th	10.5	8.4	0.42	6.0	5.7	4.8
U	2.5	2.2	0.05	1.25	1.3	1.25
Pb	15	22	13	12	15	10
Zr	240	193	202	181	210	100
Hf	5.8	3.8	3.6	5.8	4.7	3.0
Nb	25	6	5	10	13	11
La	30	36	22	29	28	19
Ce	64	69	44	61	57	38
Nd	26	30	18.5	23	23	16
Sm	4.5	4.4	3.3	4.9	4.1	3.7
Eu	0.88	1.09	1.18	1.29	1.09	1.1
Tb	0.64	0.41	0.43	0.65	0.53	0.64
Tm	0.33	0.14	0.19	0.25	0.24	0.32
Yb	2.2	0.76	1.2	1.5	1.53	2.2
Lu	0.32	0.1	0.18	0.24	0.23	0.30
Y	22	9	7	15	14	22
Weight	8	3	9	4		
Element ratios						
K/Rb	249	236	755	302	286	296
Rb/Sr	0.314	0.128	0.019	0.110	0.121	0.105
Ce _N /Yb _N	7.4	23	9.3	10.3	9.5	4.4

* Upper crust composition is from ref. 10.

† Middle crust composition is that of Archaean Lewisian amphibolite facies gneisses from ref. 27. The U content is calculated using a Th/U ratio of 3.8 (ref. 10) and the Lu value is obtained by extrapolation.

‡ Lower crust composition is that of Archaean Lewisian granulite facies gneisses from ref. 28. The U value is from ref. 37 and the Lu value is obtained by extrapolation.

§ The post-Archaean middle and lower crust composition is that of an average continental margin orogenic andesite. Trace element data (and K₂O, TiO₂ and P₂O₅ data) are from the average Andean andesite in ref. 25. Major element data are for the average Andean andesite from ref. 24, Table 1.

|| In calculating an average crustal composition it is assumed that the proportions of upper, middle and lower crust are 2:1:3. The upper crustal average¹⁰ is presumed to be representative of upper crust of all geological ages. The middle and lower crust are presumed to be composed of 75% Archaean material and 25% post-Archaean material (represented by the average orogenic andesite of column 4). Thus the relative weightings for upper crust, Archaean middle crust, Archaean lower crust and post-Archaean middle and lower crust become 8:3:9:4.

the deeper levels of the crust must be composed of refractory granulite-facies material.

On the basis of these observations we make the following assumptions in estimating the composition of the continental crust.

(1) That 75% of the volume of the present continental crust had been generated by the end of the Archaean, and that the composition of this crust is best approximated by average analyses of Archaean terranes.

(2) That the dominant mechanism for crustal growth in the post-Archaean has occurred through magmatism at cordilleran-type continental margins, where andesitic/tonalitic compositions are the dominant rock type.

In selecting compositions representative of the various crustal components we have taken the average upper crust estimate of Taylor and co-workers^{9,10} as being typical of the upper one-third of continental crust of all geological ages. The model takes only indirect account of the sedimentary component of the crust, which is assumed to entirely represent recycled juvenile crustal

material. For the post-Archaean middle and lower crust we have used an average continental margin andesite^{24,25} on the assumption that this composition is representative of juvenile magmatic crustal additions at continental margins. For the Archaean middle and lower crust we have used averages for amphibolite-facies and granulite-facies terranes from the Lewisian of north-west Scotland²⁶⁻²⁸. The lower crust is of fundamental importance in determining the bulk composition of the continental crust. The andesite model^{9,10} derives an inferred composition for the lower crust which is neither compatible with geophysical data nor with the observed composition of terranes representative of the deep crust. We believe that the composition of the lower crust is best constrained by consideration of Archaean granulite-facies terranes; first, because crustal growth rates indicate the volumetric dominance of the Archaean rocks; second, because Archaean high-grade terranes record *P-T* conditions appropriate for deep levels of the continental crust^{29,30}; and third, because only Archaean granulite-facies terranes display the severe depletion in radioactive heat producing elements, and low heat pro-

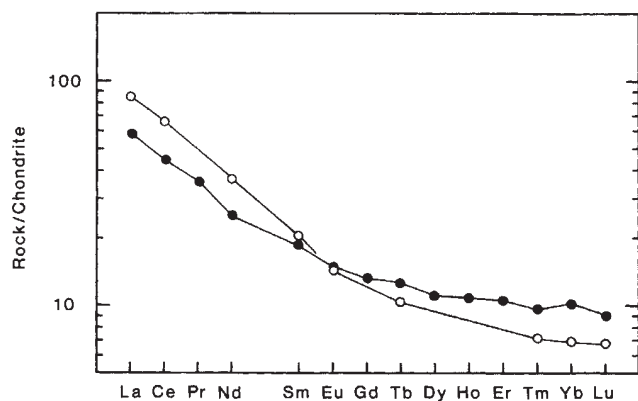


Fig. 1 Chondrite normalized³⁸ abundances of the REE in the estimated continental crust compositions of Table 2. ●, 'Andesite' model (column 6, Table 2); ○, new model presented here (column 5, Table 2).

duction, required by heat flow data²¹⁻²³, whereas Proterozoic and Phanerozoic granulites show minor or no depletion in these elements^{29,31}. The compositions (and relative weightings) of the crustal components which we have used in calculating a composition for the continental crust are given in Table 2.

In contrast to the andesite model (column 6, Table 2), our average continental crust (column 5, Table 2) is significantly more siliceous and closer, in terms of major element chemistry, to previous crustal estimates (Table 1). Several trace elements have similar abundances in the two estimates (Cr, Ni, Th, U, Nb, Y and the heavy rare-earth elements (HREE): see columns 5 and 6, Table 2), but the abundances of the remaining elements (Rb, Sr, Ba, Pb, Zr, Hf and the light rare-earth elements (LREE)) are appreciably higher in our estimate. In particular, the new crustal estimate has a much more fractionated rare-earth element pattern as illustrated in Fig. 1. There is no significant Eu anomaly. The enhanced LREE enrichment is more consistent with modelling of Nd isotopes in the crust-mantle system³². The differences in minor and trace-element abundances between the new estimate and the andesite model are further illustrated in Fig. 2, where the abundances of the elements are normalized to an average primordial mantle composition³³. On first consideration, the profiles of the two patterns in Fig. 2 are similar, with generally increasing normalized abundances from right to left. However, this pattern is superimposed on large negative 'anomalies' for the elements Nb, P and Ti. The relative depletion in these elements is a common feature of subduction related magmas^{34,35}, and often attributed to stabilization of accessory phases during magma genesis in hydrous conditions³⁴. This feature is also typical of the dominant tonalitic gneisses and plutons in segments of Archaean crust^{26,36}, and would lead us to extend to the Archaean our assumption that crustal growth occurs dominantly at cordilleran-type continental margins. However, Archaean tonalitic gneisses and plutons display a strong depletion in the HREE which is not generally evident in otherwise compositionally similar post-Archaean rocks^{26,36}, a feature explicable in terms of partial melting of subducting oceanic lithosphere in Archaean, but not post-Archaean, times²⁶. The andesite model takes no account of any such temporal variation in the composition of juvenile crustal additions, nor of temporal variation in the chemistry of the mantle. It is the strong HREE depletion in the Archaean compositions of Table 2 which imparts the high Ce_N/Yb_N ratio to our crustal average.

The abundances of the radioactive heat-producing elements Th, U and K are higher in our average crust than in some previous estimates^{10,23}, and consequently heat-production is higher in our model ($0.95 \mu\text{W m}^{-3}$ compared with a range $0.73-0.91 \mu\text{W m}^{-3}$; refs 1, 23). For an average crustal thickness of 35 km, our estimate produces a mean heat flow of 33 mW m^{-2} . Adopting appropriate values for the abundances of trace elements in the primordial mantle³³, the continental crust composition presented here contains between 40 and 50% of the Earth's

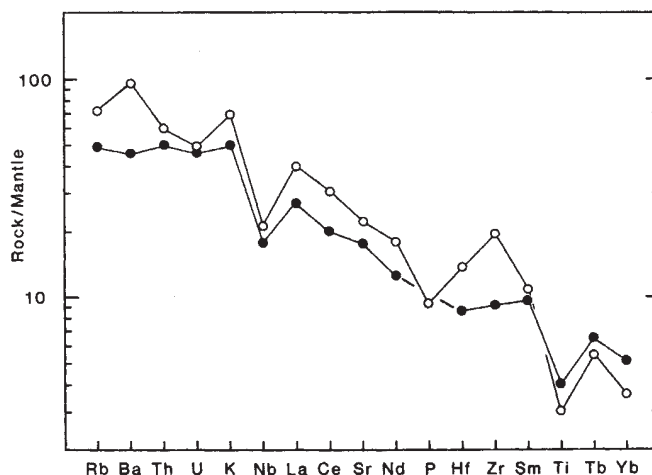


Fig. 2 Primordial mantle normalized³³ abundances of selected trace elements in the estimated continental crust compositions of Table 2. Symbols as in Fig. 1.

radioactive heat-producing elements.

An important feature of the continental crust is the depletion in high field strength elements (Fig. 2). Although there is some evidence to suggest that average normal MORB may display a slight positive Nb (and Ta) anomaly³³, the magnitude of this anomaly is insufficient to compensate for the strong depletion in these elements in the crust. That the MORB source alone is the complementary reservoir to the continental crust is, therefore, an oversimplification. Ocean island and continental alkali basalts, however, are generally markedly enriched in Nb relative to the LREE and large-ion lithophile elements³³. Clearly, trace element and isotope models for the evolution of the crust-mantle system require consideration of at least three components: the continental crust and two, or more, mantle reservoirs. We believe that the estimated composition for the continental crust presented here is more realistic than hitherto available, and will allow better constraints to be placed on models for mass transfer within the crust-mantle system.

Received 30 March; accepted 19 June 1984.

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Fluorescent bands in massive corals record centuries of coastal rainfall

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Massive coral colonies on the Great Barrier Reef grow outwards at 5–25 mm yr⁻¹ (ref. 1). Skeletal density varies seasonally in all massive corals from the Great Barrier Reef and an exact temporal record of growth can be obtained by X-radiography of appropriately cut sections of the colony^{2,3}. Environmental influences on coral growth have been described from such analyses^{4,6}. This is the first report from the skeletons of massive corals of yellow green fluorescent bands which appear under long-wave UV light. Such fluorescence is confined to corals growing within 20 km of the shore and is not present in massive corals from mid- and outer shelf reefs of the central Great Barrier Reef region. The timing, width and intensity of the fluorescent bands correlate strongly with summer, monsoonal rainfall and coastal runoff. Large colonies, several centuries old, can provide long records of the strength and periodicity of terrestrial runoff to the tropical nearshore environment. Such records are potentially important to climatology, meteorology, agriculture, civil engineering and management of the Great Barrier Reef.

Cores were drilled from very large colonies of *Porites* spp. on the lee of Pandora Reef, a 60 hectare platform reef lying in 10 m of water at 18°51'S, 146°26'E. One of these cores, comprising the whole vertical depth of the colony (1.8 m), was sectioned and analysed by X-radiography. This revealed the coral to be 118 yr old, with a mean annual growth rate of 15 mm yr⁻¹. X-radiographs of the core were compared with photographs of the same core fluorescing under long-wave UV light. Periodic yellow-green fluorescence of bright but variable intensity was exhibited within the high density regions of the annual density bands. On the Great Barrier Reef the highest skeletal density of massive corals is deposited in summer¹. The core was illuminated with long-wave UV light and stepped in 0.5 mm increments past the tip of an optical fibre using a milling machine bed. Fluorescence in the skeleton was measured with the optical fibre connected to the emission port of a Turner Model 430 spectrofluorometer. Output voltages from the spectrofluorometer were recorded on an HP 3497A Data Acquisition Unit connected to a HP85B computer. These data were converted into a monthly time series by comparison with the temporal record obtained from X-radiographs of the core, using previously developed techniques¹.

Outflow from the Burdekin River⁷ was correlated with the intensity of fluorescent banding in the coral core. Pandora Reef lies in the path of buoyant patches of the flood plume of this large river⁸, and the records of Burdekin flow are used here as a proxy indicator of terrestrial input to the seawater around Pandora Reef. The Burdekin flows into the sea 135 km south of Pandora Reef. It drains about 130,000 km² of the adjacent land-mass with annual runoff values varying by two orders of magnitude⁸. Most of the annual discharge occurs as floods (1–3 per year) between January and March⁸. The river plume flows northward along the coast, and does not reach the mid- or outer shelf reefs⁹.

The close synchrony of deposition of fluorescent material in the coral skeleton with the outflow of the Burdekin River is evident in Fig. 1. Their interdependence was quantified by cross-correlating the cube of the fluorescence intensity with Burdekin River flow. Maximum square cross-correlation of 0.80 (95% confidence interval = 0.69–0.87, $n = 62$) was obtained for zero time lag. The fluorescent bands record fluvial inputs down to the episodic level, as is demonstrated by fluorescent bands representing the February and April floods of the 1958 wet season. These appear as an inset in Fig. 1. As the width of the

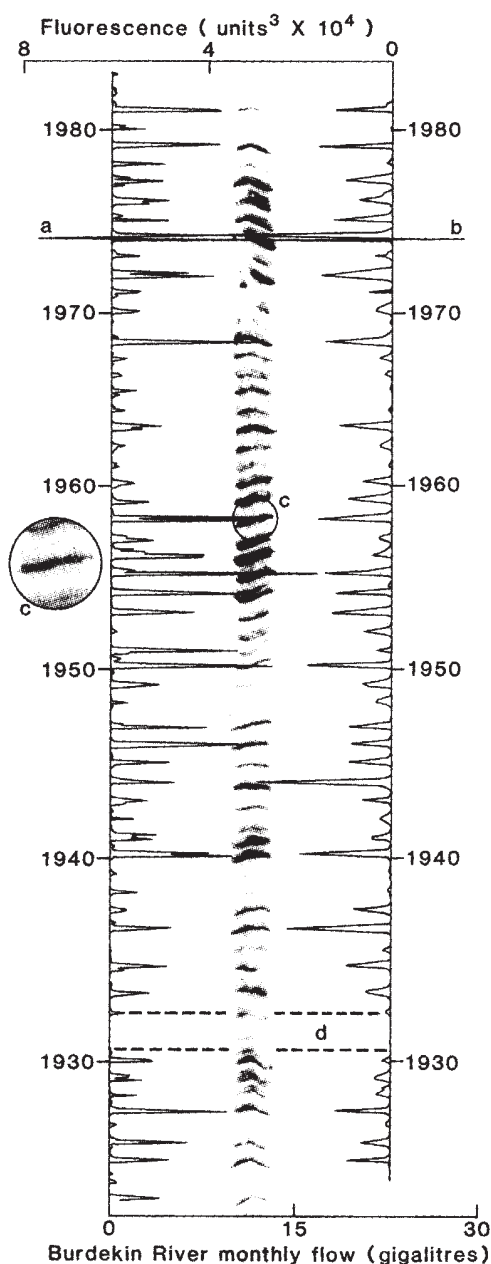


Fig. 1 Burdekin River monthly flow and measured intensity of fluorescence superimposed on fluorescent banding in 885-mm length of coral core from Pandora Reef. Coral fluorescence: a, in January 1974 mirrors large flow; b, in Burdekin River. Fine resolution of fluorescent signature is demonstrated by February and April bands in 1958 (c), enlarged in inset. The drought of 1931 is easily observed (d).

sensor attached to the spectrofluorometer imposed a 3-month limitation on resolution, instrumentation is now being constructed to resolve monthly fluorescence intensity.

Corals on mid- and outer shelf reefs are subject to monsoonal rainfall but, because of the northward flow of water in the Great Barrier Reef lagoon, they are not influenced by terrestrial runoff. The lack of annual fluorescent bands in these corals confirms that fluorescent banding in inshore corals reflects terrestrial inputs to the nearshore environment.

Fluorometric analysis of banding in scleractinian skeletons will provide a powerful tool for hindcasting the volume and periodicity of terrestrial runoff into the nearshore environment. Since this runoff is related to rainfall in the adjacent river catchments, estimates of historical rainfall will also be obtain-

able. Growth rates obtained from X-radiographic analyses of cores show that very large inshore colonies of massive *Porites* spp. are several centuries old. Thus these colonies contain long records of coastal fluvial history. The implications for regional climatology are wide. Moreover, the use of fluorescent peaks in the skeletal record in conjunction with X-radiographic analysis simplifies the task of determining seasonality of density band formation, which is a matter of controversy¹⁰. Experimental determination of the nature of the fluorescing agent in corals is being undertaken. Among other possibilities, trace inorganics of fluvial origin may be doping the aragonitic skeleton. Identification of geochemical signatures for particular river systems may contribute to our understanding of water movement over the continental shelf of the Great Barrier Reef.

I thank many of my colleagues from the Australian Institute of Marine Science for their assistance, especially D. Barnes, B. Chalker and J. C. Andrews. This is contribution no. 236 from the Australian Institute of Marine Science.

Received 13 April; accepted 11 June 1984.

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Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 of mice

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Development and fertility in the mouse are known^{1,2} to be influenced by loci mapped to the *T/t* complex of chromosome 17. Recent evidence suggests that one or more genes near this region may also be associated with sex determination. Washburn and Eicher³ recently reported partial to complete sex reversal with the *T^hp* deletion on some genetic backgrounds and suggest that this result may be due to a primary sex-determining locus (*Tas*) that is closely linked to, or a part of, the *T* locus. Sex-specific, Bkm (banded Krait minor satellite DNA)-related sequences are known to have autosomal as well as heterogametic sex chromosomal copies, but specific regions of autosomal localization have not been described^{4,5}. We now demonstrate the presence of chromosome Y-related DNA sequences on proximal chromosome 17 in *Sex-reversed* (*Sxr*) and normal mice using *in situ* hybridization of mitotic chromosomes with ³H-labelled pCS316 (ref. 4), a probe that shows major hybridization to the proximal portion of the mouse chromosome Y^{6,7}. These data, and those of Washburn and Eicher³, argue for a gene(s) related to sex determination or differentiation within the proximal portion of mouse chromosome 17.

The probe used, pCS316⁴, is cloned into pBR322 and represents a segment of *Drosophila melanogaster* DNA containing Bkm-related sequences. Previous studies^{4,7} have shown that Bkm-related DNAs such as pCS316 hybridize to precisely the same sequences as Bkm in mammals and reptiles and that they are concentrated within the sex-determining chromosome of many eukaryotes⁴. Therefore, it has been proposed that Bkm and its related sequences represent a conserved sex chromosome-associated nucleotide sequence^{4,7} which may be expressed^{8,9}.

pCS316 was labelled and hybridized to chromosome spreads from normal and *Sxr* mice. *Sxr* is a sex-limited condition which causes XX mice to appear as phenotypic males. The following six genotypes were analysed: three genotypes (a X/X *Sxr* male, a X/Y *Sxr* carrier male and a X/X female) were fetal siblings derived from the mating of a normal random-bred (CD-1) female to a 129/SvPas-Ta-*Sxr* X/Y carrier male, two other genotypes (a X/X *Sxr* male and X/Y carrier male) were of the 129/SvPas-Ta-*Sxr* inbred strain, and the final genotype was composed of random-bred (CD-1) normal males. At least 50 metaphase spreads from each of the six genotypes ($n > 300$) were analysed. Regardless of the genetic background, roughly 60% of the metaphases studied showed grain concentrations in bands B to D of chromosome 17. Figure 1 shows these results, with the corresponding idiogram¹⁰.

To determine whether hybridization of pCS316 to autosomal DNA was due to sequence homology or nonspecific binding, the frequency of hybridization within a given chromosome region was calculated for chromosome 17 with chromosome 16 as a control (Fig. 3). Selection criterion for metaphase quantitation was based on a display of hybridization to at least one chromosome of the pair. Grains appeared most frequently in two distinct regions of chromosome 17. One region, extending from 17A3 to the B–C interface, showed grains in 71% of the chromosomes examined. The other region extended from 17D to the D–E1 interface and showed the presence of grains in 38% of the chromosomes. In contrast, the region of greatest binding on chromosome 16, the telomeric end, showed grain localizations in only 7% of the chromosomes studied. The data indicate that the grains seen in the 17B–D region of chromosome 17 in Fig. 1 are due to the specific hybridization of pCS316 to homologous sequences on the chromosome.

As expected, the X chromosome of presumed paternal origin (*X^{Sxr}*) demonstrated unequivocal hybridization at its distal terminus in X/X *Sxr* males⁷. We have also discovered another hybridization site near the XA5 region in both the *X^{Sxr}* and the normal maternal X. Figure 2a, from a X/X *Sxr* male, illustrates a typical G-banded, fetal liver metaphase spread. The *X^{Sxr}* and its homologue are shown in Fig. 2b with the standard idiomorph¹⁰. The Y chromosome of the X/Y *Sxr* carrier male possessed both proximal and distal grain localization but on grain removal its morphology was similar to that of the normal Y (data not shown). This observation confirms the findings of Evans *et al.*¹¹ that the *Y^{Sxr}* chromosome possesses a distal body but otherwise is cytologically normal. The Y chromosome from normal random-bred males showed the expected proximal hybridization^{6,7} to bands A to B. On most autosomes, as for chromosome 16, grain localizations were infrequent and random. However, in addition to chromosome 17, we observed another autosome, identified as chromosome 4 (K. K.-M. and R. P. E., unpublished), which seemed to show hybridization to its mid-region in over 50% of the metaphases examined.

Structural and behavioural characteristics of chromosome regions containing concentrations of the evolutionarily conserved Bkm-related sequences suggest that these (or closely linked sequences) are connected with the determination of sex⁴. For example, in the X/Y *Sxr* male, when a region of chromosome showing dense pCS316 hybridization is transferred from the distal end of the *Y^{Sxr}* to an X chromosome during meiosis, X/X male progeny result. In terms of chromosome 17, the amount of pCS316 hybridization (as judged from grain density) is considerably less than that observed on the Y chromosome. However, it is probable that the probe is pairing with a similar family of DNA sequences. Furthermore, the finding by Washburn and Eicher³ that a *T*-associated sex reversal trait, *Tas*, results in partial to complete sex reversal in *Tas*/+;XY individuals on certain genetic backgrounds implicates the presence of sex-related gene(s) within the proximal portion of chromosome 17. A locus near this region, which may have a role in sex determination, is the histocompatibility-Y expression locus, *Hye* (refs 12, 13), for which a previous analysis of recombinants demonstrated a location between the *H-2* and *T*

Fig. 1 Representative pairs of chromosomes 17 after *in situ* hybridization with the pCS316 probe followed by G-banding in Wright's stain. *a*, Chromosome 17 from two X/X *Sxr* males. Chromosome pairs 1–3 were obtained from the X/Y *Sxr* male progeny of a CD-1 $\times$ 129/SvPas-Ta-*Sxr* X/Y carrier mating. The mitotic spreads were prepared from fetal liver. Chromosome pairs 4–6 were obtained from a X/X *Sxr* male of inbred 129/SvPas-Ta-*Sxr* stock and prepared from adult bone marrow. *b*, *c*, Chromosome 17 pairs from X/Y *Sxr* carrier and X/X female, respectively. The chromosomes, prepared from fetal liver, were obtained from the fetal siblings of a CD-1 $\times$ 129/SvPas-Ta-*Sxr* X/Y carrier male mating. *d*, Chromosome 17 from X/Y normal (CD-1) male. Bone marrow spreads from three randomly bred CD-1 males were analysed. Only one CD-1 male is illustrated here.

Methods: Mitotic spreads used for analyses were prepared from fetal livers (day 14) or adult bone marrow, as described by Eicher and Washburn¹⁰. pCS316 DNA was transfected into *Escherichia coli* DH-1. Plasmid DNA was isolated from ampicillin-treated bacteria in CsCl gradients¹⁷ and nick-translated with ³H-TTP (NEN, NEK-005) to a specific activity of 3×10^7 c.p.m. per μ g. The probe was precipitated in ethanol and resuspended in 50% formamide/ $2 \times$ SSC/ 100μ g ml⁻¹ salmon sperm DNA/ 10% dextran sulphate/ 10 mM NaPO_4 pH 7.0 at a concentration of 2.8×10^4 c.p.m. μ l⁻¹. DNA was denatured in a 100°C water bath for 5 min, then quenched on ice. The subsequent hybridization steps and autoradiography process were performed using the procedure of Harper and Saunders¹⁸. Chromosomes were stained using 0.25% Wright's stain diluted 1:3 with 0.06 M phosphate buffer pH 6.8 for 5 min as described by Chandler and Yunis¹⁹. To achieve banding, the above procedure was modified to include warming the slide sequentially at 31.5°C for 14 min, 37.5°C for 14 min, 42°C for 10 min and 53°C for 5 min in phosphate buffer. After each warming, the slide was removed from the buffer, stained for 3.5–6 min, examined and destained if necessary, by immersion first in a solution of 95% ethanol–1% HCl for 15 s and then in absolute methanol for 15 s. Samples were destained, heated and restained until satisfactory banding was achieved.

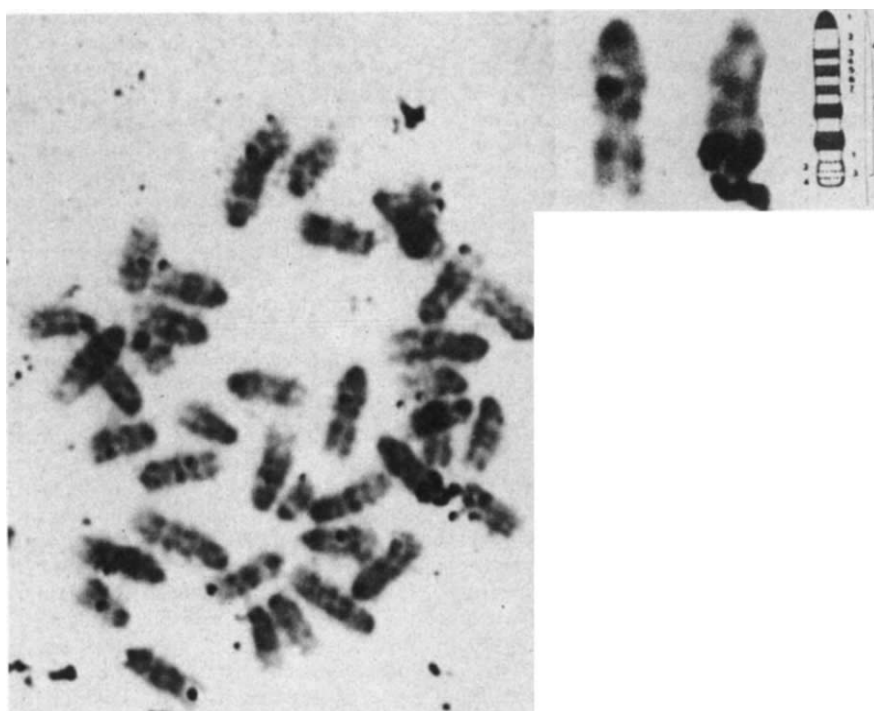
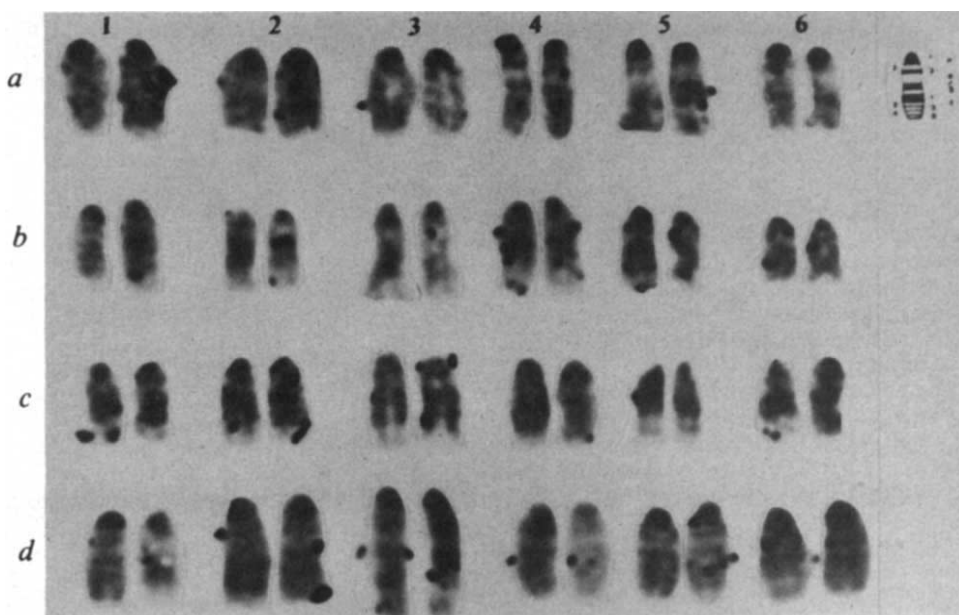


Fig. 2 ³H-labelled pCS316 hybridization to mitotic chromosomes from a sex-reversed X/X *Sxr* male. *a*, Metaphase spread from a X/X *Sxr* male. *b*, The standard idiogram of the mouse X chromosome¹⁰ and the two X chromosomes from the metaphase shown in *a*.

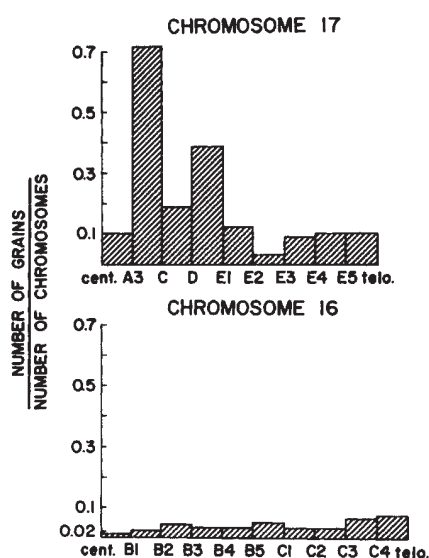


Fig. 3 Histogram frequencies of grain distribution within given chromosome regions on chromosomes 17 and 16, respectively. Normal male, X/X *Sxr* male, X/Y *Sxr* male and X/X female metaphases exhibiting hybridization to chromosome 17 were chosen for quantitation. Chromosome regions shown in Fig. 1 are defined in terms of boundaries (that is, for chromosome 17, A3–C represents the chromosome region extending from A3 to B and terminating at the B–C interface) (see Fig. 1 for idiogram of chromosome 17). Frequencies were determined by dividing the total number of grains per chromosome region by the number of chromosomes examined ($n = 94$).

complexes, closer to the latter¹³. Although *Hye* was originally found to affect the amount of transplantation H–Y, it has been found also to alter the amount of serological H–Y¹⁴. To the extent that levels of this antigen have a role in sex determination, a decreased expression (as may occur in the *T^{hp}* deletion) might cause hermaphroditism or sex reversal on some genetic backgrounds.

Intriguingly, translocation studies have mapped a large portion of the *T/t-H-2* complex¹⁵, and therefore most probably the *Hye* locus¹³, within a region that shows significant hybridization to the sex-determining-related pCS316 DNA. In light of the previous unsuccessful attempts to locate a structural gene for the H–Y antigen, one might speculate that the H–Y structural locus may itself be located within the *T/t-H-2* complex of chromosome 17 and perhaps, more specifically, in its pCS316-related sequences.

We thank K. W. Jones for providing pCS316 DNA and Michael P. Rosenberg for technical assistance and a critical reading of the manuscript. This work was supported by NIH grant HD 11738.

Received 6 February; accepted 1 June 1984.

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Chromosomal localization of the human proto-oncogene *c-ets*

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E26 is an acute leukaemia avian retrovirus which induces myeloblastosis and erythroblastosis *in vivo* and transforms erythroblasts and myeloblasts *in vitro*^{1–3}. It contains the oncogene *v-myb* (ref. 4), first described for avian myeloblastosis virus (AMV), as well as a second specific nucleotide sequence, *v-ets* located 3' to *v-myb* (refs 5, 6). We have reported that *v-ets* has a cellular counterpart (*c-ets*) in chicken and human DNA⁵. Now, using two independent methods—hybridization with human *c-ets* probe of sorted chromosomes and *in situ* hybridization—we report the localization of the *ets* locus on human chromosome 11 at bands q23–q24. This finding may be important, as specific breakpoints around this position have been reported for human malignancies such as acute monocytic leukaemia and Ewing's sarcoma.

Recombinant phages containing nucleotide sequences (*c-ets*) related to the *v-ets* sequences of E26 retrovirus were isolated from a genomic library of normal human DNA⁷. As shown in Fig. 1, a human *c-ets* probe was derived from one such phage, λ *ets* H-3, as a 5.4-kilobase (kb) *Eco*RI fragment which hybridized specifically to a 8.6-kb *Eco*RI genomic fragment of human DNA also detected with a *v-ets* probe. This 5.4-kb fragment, containing no repetitive DNA, was inserted into the *Eco*RI site of plasmid pKH47 to yield the probe used for hybridization experiments on human chromosomes.

Hybridization experiments using sorted chromosomes indicated that the human *c-ets* locus was located on the long arm of chromosome 11. Metaphase chromosomes were separated into nine peaks, immobilized on nitrocellulose filters⁸ and hybridized with the *c-ets* probe. With the Raji Burkitt cell line, shown by cytogenetic data to contain normal chromosomes 11,

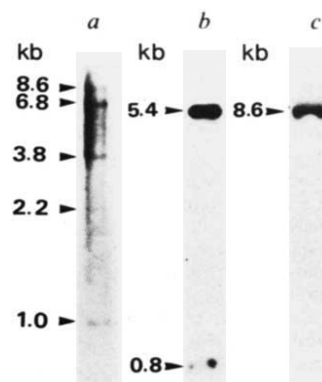


Fig. 1 Characterization of the probe *c-ets* used in hybridization experiments. Recombinant phage λ *ets* H-3 DNA was digested with *Eco*RI, separated by agarose gel electrophoresis and hybridized with ³²P-labelled *v-ets* probe. Two *Eco*RI fragments (5.4 and 0.8 kb, respectively) were detected by autoradiography (b). Southern blots of placental DNA restricted by *Eco*RI were hybridized in low-stringency conditions with *v-ets* nick-translated probe (a) or in high-stringency conditions with 5.4-kb *Eco*RI fragment of phage *ets* H-3 (c).

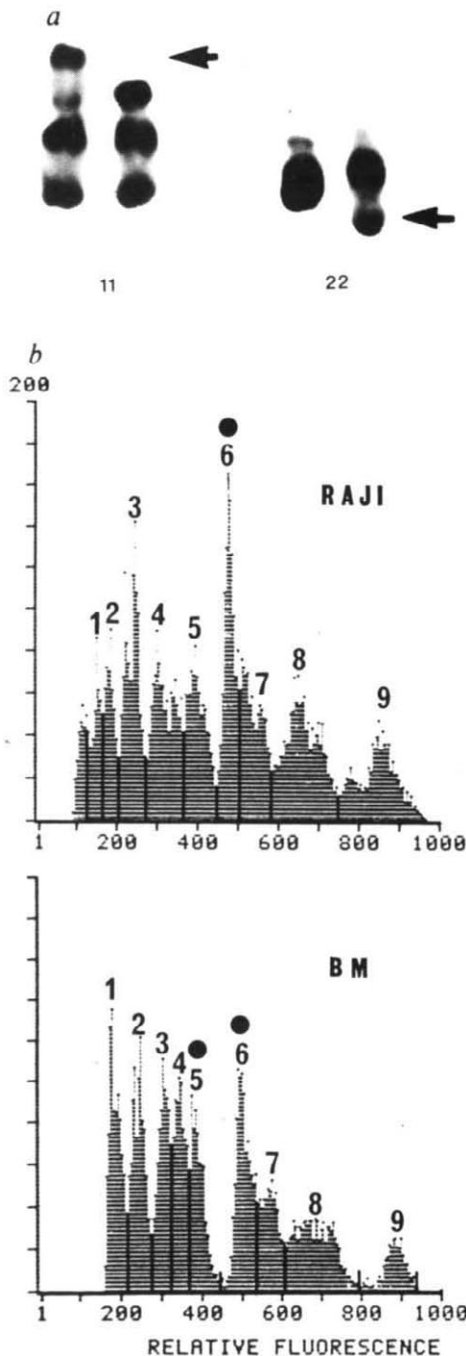


Fig. 2 Localization of the *c-ets* gene on a flow karyotype of Raji and BM cells. *a*, Partial karyotype of the BM cell line, showing the balanced translocation $t(11;22)(p11;q22)$ (R-bands). *b*, Relative fluorescence of the flow karyotype of Raji and BM cells. The number of chromosomes is plotted as a function of the relative fluorescence. Numbers indicate the position of the nine different fractions. Black dots show the peaks with radioactive signals obtained by hybridization with the *c-ets* probe.

Methods: The flow karyotypes were carried out using isolated chromosomes in polyamine buffer with an Ortho H50 cytofluorograph monitored by an Ortho 2150 computer. Nine different regions were selected on the flow karyotype (the bars show the limits of the gates) and the chromosomes were directly collected on nitrocellulose filters⁸. Denaturation was achieved by soaking in 0.5 M NaOH, 1.5 M NaCl for 5 min followed by neutralization in 0.5 M Tris-HCl (pH 7.4), 1.5 M NaCl. After baking, all filters of the same experiment were hybridized ($3\times$ SSC, $1\times$ Denhardt's buffer, $100\mu\text{g ml}^{-1}$ denatured salmon sperm DNA, 50% formamide) with the 5.4-kb *c-ets* *Eco*RI fragment labelled with ^{32}P -dCTP (NEN). Filters were not re-used.

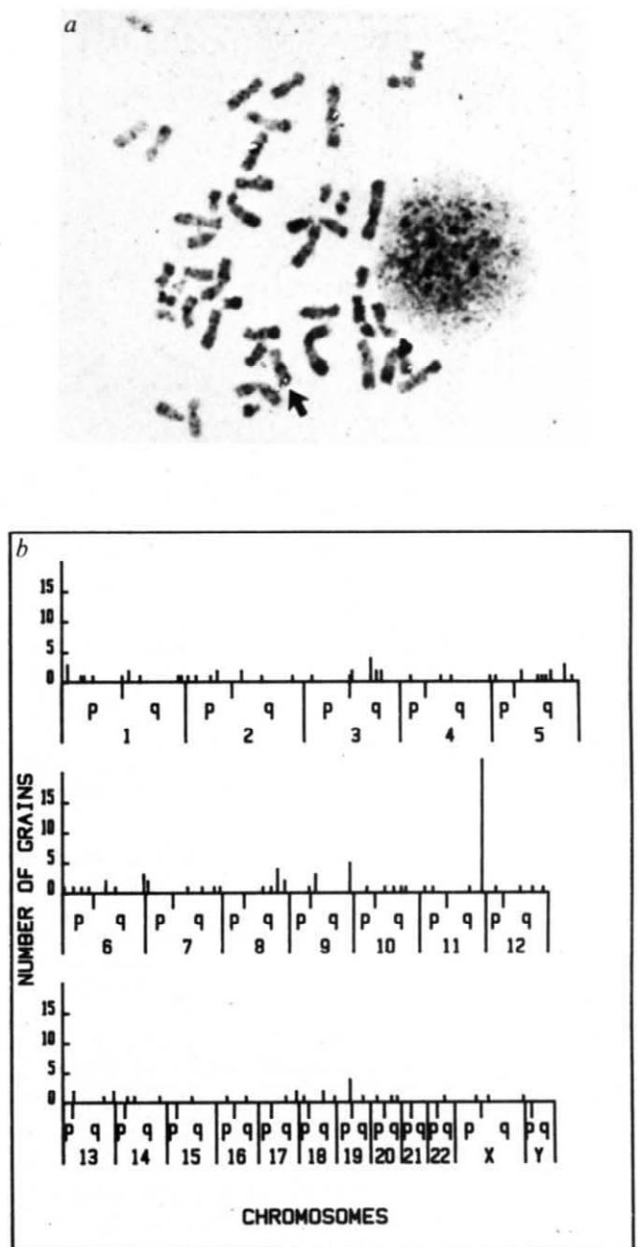


Fig. 3 Localization of the *c-ets* gene by *in situ* hybridization. *a*, Photograph of an R-banded lymphocyte metaphase spread hybridized with the *c-ets* probe. *b*, Diagram showing the grain distribution in 58 metaphases. The abscissa represents the chromosomes in their relative size proportion; the ordinate shows the number of silver grains.

Methods: Metaphase spreads were prepared using normal human male phytohaemagglutinin-stimulated lymphocyte *in vitro* cultures for 72 h, and R-banded, but staining was omitted²⁴. Total plasmid DNA containing the human *c-ets* 5.4-kb *Eco*RI fragment was nick-translated with ^3H -dCTP (110 Ci mmol^{-1} ; NEN). The techniques used for *in situ* hybridization were essentially as described by Gerhard *et al.*²⁵ The probe had a specific activity of $1.5\times 10^7\text{ c.p.m. }\mu\text{g}^{-1}$. $30\mu\text{l}$ of probe solution at 200 ng ml^{-1} in hybridization solution (50% formamide, $2\times$ SSC, 10% dextran sulphate, $100\mu\text{g ml}^{-1}$ of sonicated salmon sperm DNA) were placed on each slide at 37°C for 18 h. The slides were rinsed for 15 min twice in $2\times$ SSC at 55°C and then twice in $0.1\times$ SSC at 50°C . Autoradiography was performed using a standard technique (NTB3 Kodak emulsion) for 23 days before development. The slides were stained for 10 min in a 4% Giemsa solution in phosphate buffer at pH 6.8. The picture was taken by phase contrast microscopy, yielding white spots for grains on chromosome 11q (arrowed).

a positive signal was observed for peak 6, corresponding mainly to chromosomes 10, 11 and 12. From *in situ* experiments done simultaneously (see below), chromosome 11 was suspected to yield the positive signal. Analysis of the lymphoblastoid cell line BM, established from a woman carrying a balanced translocation t(11; 22) (p11; q22) (Fig. 2a), revealed two positive signals, one in peak 6, corresponding to the normal chromosome 11, and one in peak 5 (Fig. 2b), corresponding to the abnormal chromosome 11p⁻, thought by its size to be sorted with chromosomes 13 to 15.

To obtain a more precise localization of *c-ets*, we performed *in situ* hybridization experiments on chromosome spreads. Analyses of 58 metaphase spreads revealed (Fig. 3) that 17% (25/148) of all silver grains were located on human chromosome 11 ($P < 0.001$ compared with a chromosomal random distribution). Of these 25 silver grains, 22 (88%) were localized on band q24 (Fig. 3). However, because there is often a discrepancy between the R and G bands⁹, a localization at band 11q23 cannot be excluded. Secondary peaks of grains accumulated at positions 9q34 (3.4%) and 8q23 (2.7%); 8 grains (5.8%) were found between 3q11 and 3q22. The significance of these faint signals is unclear. It is unlikely that they represent *c-ets*-related loci because in our hands, extensive screening of human DNA libraries never produced more than one *c-ets* locus.

In conclusion, the human *c-ets* gene is located on chromosome 11 q23–q24. This finding indicates that although *v-ets* and *v-myb* oncogenes are adjacent in the E26 genome, their cellular counterparts are located on different human chromosomes, as *c-myb* has been assigned to chromosome 6q22–24 (ref. 10). Previous analysis of the chromosomal localization of the oncogenes of other acute leukaemia retroviruses containing two oncogenes, *erb-A* and *erb-B* for avian erythroblastosis virus (AEV), and *mil* and *myc* for MH2, have demonstrated that their human counterparts are located on different chromosomes^{11–13}. However, whereas the *erb-A/erb-B* genes of AEV and the *mil/myc* genes of MH2 are expressed as two independent transcription units, the *myb* and *ets* genes of E26 are translated from a unique mRNA⁶ as a triple fusion protein, p135^{gag-myb-ets}.

Cellular oncogene sequences have been localized at or next to chromosomal breakpoints involved in specific translocations described in human malignancies¹⁴. With respect to our localization of the *c-ets* oncogene on human chromosome 11, several rearrangements involve this chromosome at q23 and q24. The bands 11q22–24 have been found to be involved mainly in acute monocytic leukaemia, especially the undifferentiated form¹⁵. Rearrangements of band 11q23 are now thought to be the most specific chromosomal abnormality in acute monocytic leukaemia and in some cases translocations t(9;11) (p21; q23) (ref. 16) and t(11; 19) (q23; p12 or q12) (refs 17, 18) have been reported²⁰. Another specific translocation, t(4; 11) (q21; q23), has also been described in a particular form of acute leukaemia which was classified either as lymphoblastic or as immature monocytic precursor proliferation^{20,21}. Finally, a t(11;22) (q24; q12) translocation has been reported as specific for Ewing sarcoma^{22,23}.

Thus, band 11q23 and 11q24, within which we mapped the human *c-ets* locus, are involved in specific chromosomal rearrangements of well defined malignant proliferations. Experiments are in progress to investigate possible DNA rearrangements and expression of *c-ets* in these malignant diseases in correlation with cytogenetic data. Southern blot analyses of the DNAs from five different Ewing's sarcomas have so far failed to show translocation of *ets*.

We thank Professor A. Boué for providing the BM cell line; Mr R. Miglierina for his contribution to chromosome sorting with the cell sorter of the "Service Commun de Cytofluorométrie", Institut de Recherche sur les Leucémies, Paris; Dr F. Sigaux for his help in computerizing the data; and D. Leprince for the *c-ets* clone. This work was funded by INSERM, CNRS and the Association pour la Recherche sur le Cancer. C.T. is a fellow of the Ligue Nationale Française contre le Cancer.

Received 24 April; accepted 7 June 1984.

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The p21 *ras* C-terminus is required for transformation and membrane association

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The Harvey murine sarcoma virus (Ha-MuSV) transforming gene, *v-ras*^H, encodes a 21,000 molecular weight protein (p21) that is closely related to the p21 proteins encoded by the cellular transforming genes of the *ras* gene family^{1–8}. The primary translation product (prop21), which is found in the cytosol, undergoes post-translational modification⁹ and the mature protein subsequently becomes associated with the inner surface of the plasma membrane and binds lipid tightly^{10,11}. The p21 proteins have the capacity to bind guanine nucleotides non-covalently *in vitro*^{3,12}. To assess the biological relevance of these biochemical features of the protein, we have now studied a series of deletion mutants located at or near the C-terminus of the viral p21 protein. Our tissue culture studies indicate that amino acids located at or near the C-terminus are required for cellular transformation, membrane association and lipid binding.

The mutants were derived by making small deletions in a parental recombinant plasmid (Fig. 1) that contained the Ha-MuSV transforming region (the viral long terminal repeat (LTR) plus *v-ras*^H)¹³. The herpes simplex virus (HSV) thymidine kinase (*tk*) gene¹⁴ was included as a linked selectable marker for analysis of the p21 encoded by mutants that would be defective for focal transformation. Two classes (I and II) of *v-ras*^H mutants were constructed: each class I mutant coded for a truncated p21 protein; each class II mutant contained a specific in-frame deletion within p21 coding sequences located near the C-terminus and terminated translation at the normal stop codon of the protein. The approach used to generate the mutants

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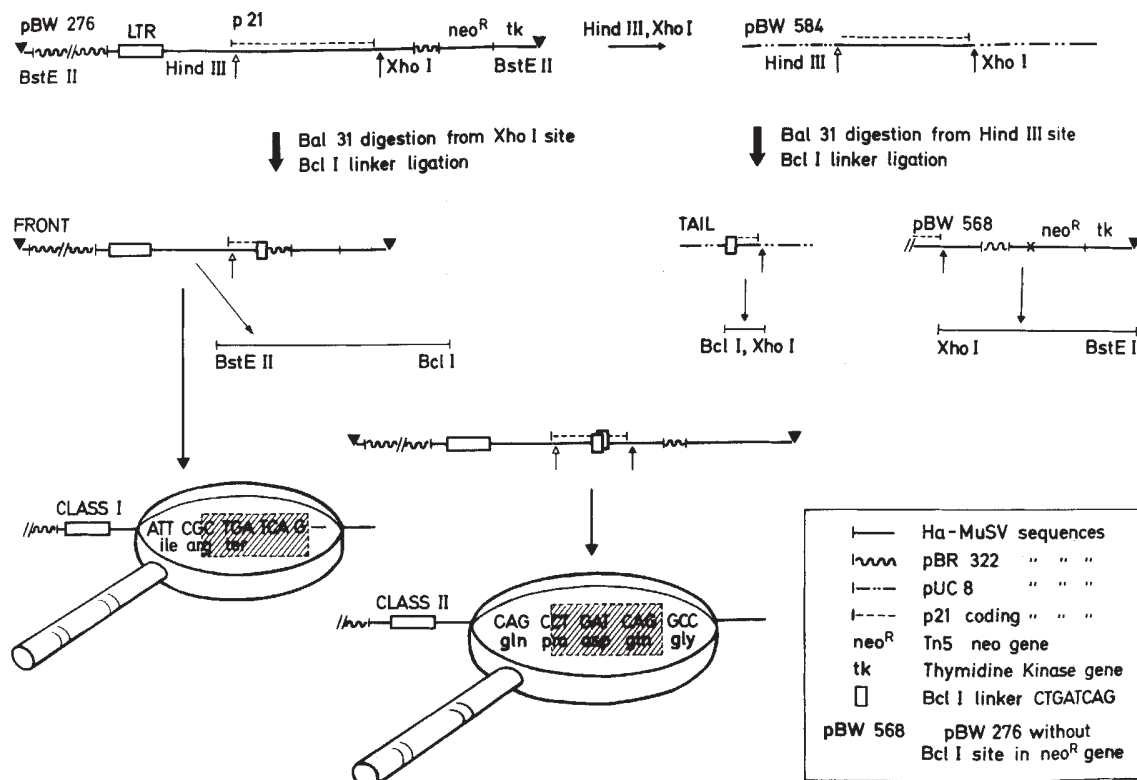


Fig. 1 Construction of mutants. The plasmids are not drawn to scale. The starting plasmid (pBW276) used for generation of the deletion mutants was composed of the transforming region of Ha-MuSV (LTR+*v-ras*^H), the HSV *tk* gene, the neomycin-resistance gene (*neo*^R) from Tn5, and the pBR322 origin of replication. It was constructed as follows: From clone H-1 (ref. 13), the *Hinc*II fragment carrying the LTR and the *v-ras*^H gene was cloned into pBR322 at the *Pst*I site after this end had been made flush with T4 DNA polymerase. The *Pst*I site located 158 base pairs (bp) downstream from the stop codon of the p21 coding sequences was converted to an *Xho*I site by insertion of an *Xho*I linker at this site. An *Eco*RI-*Sal*I fragment carrying the neomycin/kanamycin resistance gene from Tn5 (ref. 19) was inserted between the *Eco*RI and *Sal*I sites of the pBR322 portion of the plasmid. A *Sal*I fragment bearing the HSV *tk* gene (ref. 14) was then inserted in the same orientation as the p21 gene to complete pBW276. pBW276 was grown in *Escherichia coli* strain RRI (ref. 20); the *Bcl*I sites in the clone were therefore methylated and not able to be cleaved with *Bcl*I.

Methods: To generate most deletion mutants retaining various amounts of the 5' coding region of p21 (called 'front' ends), pBW276 was digested first with *Xho*I and then with exonuclease *Bal*31 for varying time periods. The DNA ends were made flush with T4 DNA polymerase and then ligated to a linker (CTGATCAG) containing the recognition site of *Bcl*I. Following digestion with *Bcl*I (which cleaved only the *Bcl*I sites in the linker because the other *Bcl*I sites in the plasmid were methylated from propagation in RRI), the DNA was ligated and digested with *Xho*I. Part of each aliquot was used to transform *E. coli* strain NF 3082 (this strain is *r*⁻*m*⁺ and carries the *dam*-3 mutation on a MC 1000 background²¹), colonies from each aliquot were first examined by restriction enzyme digestion to determine if the *Bcl*I linker was present and to estimate the extent of the deletion. For each clone used in the mutant studies, the *Hind*III-*Bcl*I fragment carrying most of the p21 sequences was cloned in M13 phage mp9 (ref. 22) and the actual deletion determined by dideoxy sequencing²³. Mutant pBW396 (mutant 3 in Table 1) was generated by this procedure alone. Its deletion began one nucleotide beyond the p21 stop codon and extended rightward for more than 150 bp. This clone also provided the front end for pBW601 (mutant 2 in Table 1). Class I mutant pBW277 (mutant 5 in Table 1) is a *Bal*31 derivative of pBW276 that did not acquire a *Bcl*I linker. Its DNA sequence happens to give a deletion where the coding region for amino acids 1-186 is unchanged and the deletion results in the juxtaposition of the codon for amino acid 186 with ACT CCT TAA from a sequence downstream from the *Xho*I site in pBW276. Some front-end deletion mutants were originally isolated after the insertion of a *Xho*I linker (instead of the *Bcl*I linker) after *Bal*31 treatment of pBW276. The *Bcl*I linker was inserted at the *Xho*I site of appropriate mutants after the plasmids had been digested with *Xho*I and mung bean nuclease so that the TGA stop codon of the *Bcl*I linker occurred in the same reading frame as the p21 protein. This resulted in the addition of CCC TGA TCAG to the p21 gene sequences. The front ends of class I premature termination mutants pBW602 and pBW603 (mutants 6 and 7 in Table 1) were constructed by this process. The plasmid (pBW584) used for the generation of p21 deletion mutants retaining varying amounts of 3'-coding sequences (called 'tail' ends) was constructed by subcloning the *Hind*III-*Xho*I fragment of pBW276 (which carries the sequences coding for amino acids 5-189 of p21 as well as 158 bp of 3'-non-coding sequences) into these sites in a modified pUC8 vector²⁴ into which a *Xho*I site had been generated by *Xho*I linker insertion at an *Eco*RI site. The tail ends were generated similarly to the front ends, by digestion with *Bal*31 from the *Hind*III site. A *Bcl*I linker was inserted at the site of deletion and each deletion was determined by sequence analysis after cloning the *Bcl*I-*Xho*I fragment in *Bam*HI-*Sal*I-digested mp9. As described below, a plasmid from which all the p21 coding sequences as well as the termination codon and 15 non-coding base pairs had been deleted serve as the tail end to 'restore' the 3'-non-coding Ha-MuSV sequences to the front end of class I mutants pBW601, 602 and 603 (mutants 2, 6 and 7 in Table 1). All class II (and restored class I) mutants were constructed by combining appropriate front and tail ends through the *Bcl*I linker so that the p21 sequences downstream from the *Bcl*I linker were in the correct reading frame. First, pBW568 (a derivative of pBW276) was constructed. In this plasmid the *Bcl*I site in the Tn5 portion of pBW276 has been removed by *Bcl*I digestion, T4 DNA polymerase filling and ligation, which did not affect the kanamycin resistance conferred by the plasmid. A three-fragment ligation reaction generated the class II (and restored class I) mutants: a *Bst*EII-*Bcl*I fragment from a front-end mutant, a *Bcl*I-*Xho*I fragment from a tail-end mutant and the *Xho*I/*Bst*EII fragment from pBW568. The recombinants were screened for the presence of a *Bam*HI site derived from pUC8 as well as for the lack of the Tn5 *Bcl*I site. The class I mutant pBW769 (mutant 4 in Table 1) was generated by recombining a front end retaining the codon for amino acid 185 (AAG) followed by the *Bcl*I linker with a tail end that contained two *Bcl*I linkers in tandem (which generated a novel *Pvu*II site). A plasmid that retained the two *Bcl*I linkers (evidenced by retention of this *Pvu*II site) was selected. The sequence of this mutant is AAG CTG ATC AGC TGA TCAG, which introduces the in-frame TGA stop codon in the second *Bcl*I linker.

resulted in each mutant encoding an insertion of one, two or three amino acids in place of the amino acids deleted from the wild-type gene; Table 1 lists the C-terminal amino acids of each mutant as inferred from DNA sequence analysis.

The DNAs were first tested for their capacity to induce focal transformation of NIH 3T3 cells. The positive controls were the starting parental plasmid (DNA 1 in Table 1) and mutants 2 and 3, which had lost bases located immediately downstream

from the nucleotide following the p21 stop codon. Both mutants transformed the mouse cells as efficiently as did the parental plasmid, indicating that the 3'-non-translated viral sequences immediately downstream from the p21 stop codon were not required for transformation. By contrast, each (class I) mutant that introduced a premature stop codon within the p21 coding sequences lacked transforming activity. These transformation-defective (td) mutants (4-7) lacked three, four or five C-terminal

Fig. 2 Lipid binding of mutant p21 proteins. Mass cultures of cells converted to TK⁺ by the mutant DNAs were metabolically labelled overnight in growth medium containing ³H-palmitic acid (1 mCi ml⁻¹)¹¹. Extracts of whole cells were prepared and precipitated with p21 monoclonal antibody YA13-259¹⁵ as described previously³. Immunoprecipitates were analysed by electrophoresis in 15% SDS-polyacrylamide gels, and bands were visualized by autoradiography for 7 days. The number above each lane refers to the mutant number as given in Table 1. Lanes C and H contain the precipitate from control cells and from cells transformed by the H-1 clone¹³ of Ha-MuSV DNA, respectively. The band that migrates just ahead of the non-phosphorylated form of viral p21 and is visualized in all lanes, including that containing the control cell precipitates, represents an endogenous form of p21 noted previously by others^{11,15}.



amino acids present in the wild type. The failure of these mutants to transform the mouse cells suggested that amino acids at or near the C-terminus might be required for p21-mediated transformation, although the td phenotype might have been a result of the one to three new amino acids at the C-terminus.

The importance of amino acids near the C-terminus was confirmed by studying the transforming activity of the (class II) mutants containing in-frame deletions within the p21 coding sequences. One set of mutants (8–13) encoded p21s with deletions beginning in His 166 and extending progressively rightward towards the C-terminus. Mutants 8–12, whose deletions extended to Ser 183, retained their transforming activity. However, mutant 13, whose deletion extended to Cys 186, did not transform the cells, suggesting that one or more amino acid(s) to the right of Ser 183 are critical for transformation. Two other in-frame mutants with smaller deletions that extended to Cys 186 (mutants 15 and 16) were also td, although their deletions were shorter than those of some transformation-competent mutants. These results confirm that one or more of the six C-terminal amino acids (184–189) of p21 are required for the protein's transforming activity. Mutant 12 indicates that amino acids 166–183 are not required for transformation.

The viral p21 proteins found in mouse cells transfected with the mutants were then analysed by immunoprecipitation¹⁵. The mutant DNAs were introduced into TK⁻ 3T3 cells¹⁴ (sensitive to hypoxanthine-aminopterin-thymidine (HAT) medium) and selected by the capacity of the intact HSV tk gene in each mutant to confer resistance to HAT medium. As expected, the mutants which had lost only non-coding sequences (mutants 2 and 3) synthesized p21 forms that were indistinguishable from those encoded by wild-type Ha-MuSV. Overnight labelling with either ³⁵S-methionine or ³H-palmitate revealed a p21 doublet which represents the two membrane-associated forms: the more slowly migrating phosphorylated form (pp21) and the faster migrating non-phosphorylated form (data are shown in Fig. 2 only for clone 2 labelled with ³H-palmitic acid). When the p21 mutants (4–11 and 14–16) were labelled overnight with ³⁵S-methionine, a doublet whose migration rate differed from that of wild-type p21 was noted for each mutant (viral p21 protein was not detected in cells transfected by mutants 12 and 13). As expected, an overnight pulse with ³²P-orthophosphate labelled only the more slowly migrating p21 species in each instance (data not shown). Overnight labelling with ³H-palmitic acid, however, distinguished between the td mutants and the transformation-competent mutants. Although the palmitic acid labelled the p21 doublet of the transformation-competent mutants, it failed to label either p21 species from the class I or II td mutants (data are shown in Fig. 2 for clones 7, 14 and 16).

These results suggested that the p21 encoded by the td mutants might not be membrane associated, in contrast to wild-type and transformation-competent class II mutants. This possibility was

Table 1 Focus formation by p21 deletion mutants of Harvey viral DNA

Mutant no.	Clone no.	Amino acid 160	Carboxy end of p21	Amino acid 189	Focus formation
1	pBW 276	VREIRQHKLRKLNPPDESGPGCMSCCKVL	ster		+
2	pBW 601	-----	-----ter		+
3	pBW 396	-----	-----ter		+
Premature termination mutants (Class I)					
4	pBW 769	-----	-----LISter		-
5	pBW 277	-----	-----TPter		-
6	pBW 602	-----	-----Pter		-
7	pBW 603	-----	-----Pter		-
Internal deletion mutants (Class II)					
8	pBW 672	----- PDQ	-----ter		+
9	pBW 679	----- PDQ	-----ter		+
10	pBW 740	----- PDQ	-----ter		+
11	pBW 739	----- PDQ	-----ter		+
12	pBW 766	----- PDQ	-----ter		+
13	pBW 754	----- PDQ	-----ter		-
14	pBW 757	----- PDQ	-----ter		+
15	pBW 756	----- PDQ	-----ter		-
16	pBW 755	----- PDQ	-----ter		-

The derivation of each mutant is given in Fig. 1. Mutants 3–5 consist of front ends; 2, 6 and 7 contain restored tail ends recombined with front ends; 8–16 contain recombined front and tail ends. Amino acids 160–189 are given for the starting clone (1); ter, termination codon. In the mutants, the symbol (–) means that this amino acid is the same as in the starting clone; a blank space means the amino acid is deleted; the letters indicate new amino acids introduced in construction of the mutant. DNAs were precipitated with calcium chloride¹⁷ and 0.2 ml was added to 35-mm dishes seeded on the previous day with 2.25×10^5 NIH 3T3 cells. NIH 3T3 DNA ($25 \mu\text{g ml}^{-1}$) was used as carrier. 0.2–0.8 μg of mutant DNA was used per dish. The cells were treated as previously described, except that dimethyl sulphoxide was not used¹⁸. Foci were counted 2 weeks later. All clones that gave rise to foci yielded $10^{2.8}$ – $10^{3.4}$ foci per μg DNA. The dishes containing the clones that failed to yield foci were kept for at least 1 month, at which time they were still negative.

tested by metabolically labelling the cells with ³⁵S-methionine, fractionating the cell homogenates into a supernatant cytosol fraction and a membrane pellet fraction, and subjecting each fraction to p21 immunoprecipitation (Fig. 3; results are shown for clones 2, 7 and 14). As has previously been demonstrated for the wild-type protein, both mature forms of the transformation-competent protein were found exclusively in the membrane (M) fraction. By contrast, both the phosphorylated and unphosphorylated forms of the td mutant-encoded protein were confined to the supernatant (S) fraction. The class I and II td mutant p21 proteins are therefore not associated with the plasma membrane. The data from the td mutants suggest further that phosphorylation of the protein can take place in the cytosol. The biological significance of the *v-ras*^H phosphorylation at Thr 59 therefore remains to be established.

Although these studies have been confined to mutations induced in *v-ras*^H, our results are probably relevant to *c-ras*^H and to the other *ras* genes, whose products also localize to the membrane and bind lipid in transformed cells (M. E. Furth and E. M. Scolnick, personal communications). The failure of the C-terminus mutants to bind lipid and associate with the membrane suggests that amino acids in this region mediate lipid binding which may help anchor the protein at the membrane. The transformation-defective nature of these mutants further

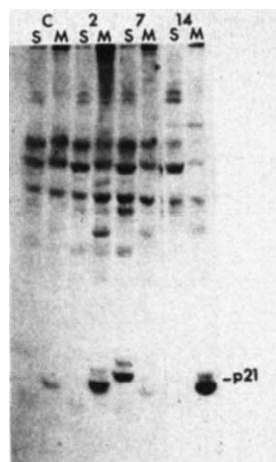


Fig. 3 Fractionation of mutant p21 proteins. Cultures converted to TK⁺ by the mutant DNAs were metabolically labelled overnight with ³⁵S-methionine (250 μ Ci ml⁻¹). Hypotonic swelling of the cells was followed by homogenization and low speed centrifugation to remove nuclei. The supernatant was then fractionated by centrifugation at 100,000g for 30 min into a pellet particulate fraction containing the plasma membranes and a supernatant cytosol fraction²⁵; this procedure has been used previously to separate cytosol-associated p21 from the membrane-associated mature p21 forms (ref. 9). The fractions were then precipitated with monoclonal p21 antibody YA6-172 as in Fig. 2. Immunoprecipitates were analysed by electrophoresis in 15% SDS-polyacrylamide gels, and bands were visualized by autoradiography for 4 days. Lanes S and M contain precipitates from the supernatant and membrane (pellet) fractions, respectively. The number above each pair of lanes refers to the mutant number given in Table 1. The pair labelled C contain the precipitate from control cells. Although mutant 7 encodes a truncated p21 protein, it migrates more slowly than wild-type p21 protein because of defective processing (B.M.W. *et al.*, manuscript in preparation).

indicates that this anchoring to the membrane is important for the biological activity of p21. (Similar conclusions have recently been reached by M. O. Weeks and E. M. Scolnick (personal communication), who have studied a td *v-ras*^H gene that has an undefined lesion.)

Of the six C-terminal amino acids, only Cys 186 is present in all known *ras* genes, although conservative changes are found at amino acid 187 (valine or isoleucine) and 188 (leucine, isoleucine or valine)^{8,16,26}. The possible importance of Cys 186 was suggested experimentally by td mutant 4, which may be viewed as encoding a protein from which only Cys 186 has been deleted. Preliminary data indicate that a point mutant substituting Ser for Cys 186 has the td phenotype (B.M.W. *et al.*, unpublished data). We speculate that Cys 186 permits membrane association and lipid binding because the lipid is bound through this cysteine residue. However, the presence of the cysteine is probably not sufficient for transformation competence, because a mutant (5) with the inferred protein sequence Cys 186 followed by Thr and Pro is td. We therefore suggest that both Cys 186 and downstream amino acids may be required for transformation.

The extraordinary sequence divergence near the C-terminus of the three biologically active mammalian *c-ras* genes (*ras*^H, Kirsten-*ras* and neuroblastoma-*ras*), spanning amino acids 165–189, has fostered speculation that each *c-ras* gene subserves distinct physiological functions that depend in part on this heterogeneity^{6,8}. This possibility is strengthened by the excellent conservation of even this region between human and rodent *ras*^H genes (refs 2, 4, 5 and M. Ruta and R. Dhar, personal communication). The data obtained with mutants 8–12 indicate, however, that this region is not required for transformation of NIH 3T3 cells. These results therefore suggest that the oncogenic

action of p21 may be distinct from the hypothetical physiological activities involving this region of sequence divergence.

We thank Niels Fiil, Bjorn Nexø, Maureen Weeks, Edward Scolnick, Sandra Ruscetti and Henry Krutzsch for helpful discussions, Marilyn Lander for technical assistance and Niels Fiil for the *neo*^R plasmid and strain NF 3082. B.M.W. is a Senior Fellow at the University of Copenhagen.

Received 24 February; accepted 7 June 1984.

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Activation of c-Ha-ras-1 proto-oncogene by *in vitro* modification with a chemical carcinogen, benzo(a)pyrene diol-epoxide

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Chemical carcinogenesis generally proceeds via the formation of strongly electrophilic reactants, termed ultimate carcinogens¹. The observation that many ultimate carcinogens are potent mutagens² and the results of studies on the covalent binding of carcinogens to cellular macromolecules^{3–5} suggest that tumour initiation results from mutations arising from the binding of ultimate carcinogens to DNA. Recently, gene transfer experiments have shown that some tumours contain activated oncogenes which are members of the *ras* gene family (reviewed in refs 6, 7) and which differ by single base pair substitutions from their non-transforming counterparts, the proto-oncogenes (see, for example, refs 8–11). Here we have used clones of the c-Ha-ras-1 proto-oncogene to show that reaction *in vitro* with an ultimate carcinogen generates a transforming oncogene when the modified DNA is introduced into NIH 3T3 cells. As DNA is the only cellular macromolecule present in the reactions, our experiments also show that reaction of an ultimate carcinogen with DNA alone can lead to the induction of mutations in mammalian cells.

For these experiments we treated the DNA *in vitro* with *r*-, *t*-8-dihydroxy-*t*-9,10-oxy-7, 8, 9,10-tetrahydrobenzo-(a)pyrene (*anti*-BPDE), the major ultimate carcinogen of benzo(a)pyrene

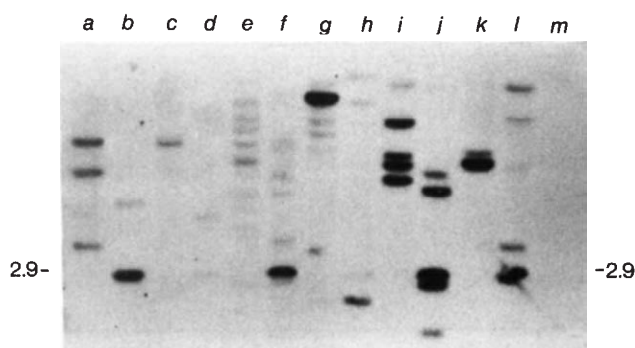


Fig. 1 Southern blot showing presence of human c-Ha-ras-1 sequences in *EcoRI*- or *SstI*-digested DNA from primary foci arising after the transfection of NIH 3T3 cells with pHa-1 containing the c-Ha-ras-1 proto-oncogene treated with *anti*-BPDE. Lane a, focus 401 (*EcoRI*); lane b, 401 (*SstI*); lane c, focus 414 (*EcoRI*); lane d, 414 (*SstI*); lane e, focus 415 (*EcoRI*); lane f, 415 (*SstI*); lane g, focus 417 (*EcoRI*); lane h, 417 (*SstI*); lane i, focus 403 (*EcoRI*); lane j, 403 (*SstI*); lane k, focus 408 (*EcoRI*); lane l, 408 (*SstI*); lane m, NIH 3T3 (*EcoRI*).

Methods: 20 µg of DNA were digested to completion with either *EcoRI* or *SstI*, electrophoresed on a 0.8% agarose gel and transferred to nitrocellulose. The filter was hybridized at 42°C in 3 × SSC, 50% formamide, 20 mM sodium phosphate, 1 × Denhardt's solution, 50 µg ml⁻¹ denatured sonicated salmon sperm DNA, 10% dextran sulphate with 10⁷ c.p.m. of a nick-translated ³²P-labelled probe of the 2.9-kb *SstI* fragment of pHa-1 (ref. 16). The filter was washed in 0.1 × SSC, 0.1% SDS at 60°C and autoradiographed at -70°C.

Table 1 Effect of reaction with *anti*-benzo(a)pyrene diol-epoxide (*anti*-BPDE) on transforming activity of pEJ

DNA/ <i>anti</i> -BPDE ratio	% Transforming activity remaining
4:1	0.6
8:1	2.0
15:1	14
80:1	100
800:1	100
1:0	100 (= 3,000 foci µg ⁻¹)

pEJ plasmid DNA (10 µg in 20 µl 1 mM Tris-0.1 mM EDTA, pH 7.6), consisting of supercoils (form I) and relaxed circles (form II) in a ratio of ~2:1, was treated with solutions of *anti*-BPDE (in 10 µl ethanol) and incubated at 37°C for 2 h. The reaction mixture was then diluted with Tris-EDTA (20 µl) and extracted five times with 80 µl ether. High-molecular weight normal mouse DNA (100 µg) was then added and the DNA precipitated with ethanol. Aliquots of redissolved DNA containing 10–15 ng plasmid DNA were added to 20 µg normal mouse liver DNA, precipitated with calcium phosphate using the method of Wigler *et al.*³⁰, and applied to 100 mm-diameter dishes seeded the previous day with 1.3 × 10⁵ NIH 3T3 cells in 10 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum. After exposure to the co-precipitate for 20–22 h, the plates were washed and maintained in DMEM supplemented with 5% (CS). The culture medium was changed every 3–4 days and foci scored after 11–12 days.

metabolism (reviewed in ref. 12), which is a potent mutagen in bacterial¹³ and mammalian cells¹⁴. To determine suitable reaction conditions between *anti*-BPDE and DNA that would allow sufficient mutations to occur without destroying the integrity of the gene, we examined the effect of increasing doses of *anti*-BPDE on the transforming activity of a plasmid, pEJ, containing the *Bam*HI fragment of the c-Ha-ras-1 oncogene cloned from EJ/T24 bladder carcinoma cells inserted into pBR322 (ref. 15). Table 1 shows that with a reaction ratio of 80:1 DNA/*anti*-BPDE, no decrease in transforming activity could be detected. At ratios less than 80:1 significant reductions in transforming activity were observed.

Two plasmids containing the coding sequence of the c-Ha-ras-1 proto-oncogene were treated with *anti*-BPDE. One of these, plasmid pHa-1 (ref. 16), contains the 2.9-kilobase (kb) *SstI* fragment that encompasses the coding sequence of the c-Ha-ras-1 gene as well as a potential cap site, Goldberg-Hogness box and CAT upstream signal^{17,18}. The 2.9-kb *SstI* fragment does not contain a possible enhancer element located upstream of the promoter¹⁸, but the requirement for this possible enhancer may be circumvented in pHa-1 by the presence of part of the long terminal repeat (LTR) of Harvey murine sarcoma virus, included in the vector in order to provide the *SstI* site. Unlike constructions that contain a complete LTR¹⁹, pHa-1 does not induce foci when transfected into NIH 3T3 cells. The second plasmid, pEC, contains an insert in pBR322 of the 6.6-kb *Bam*HI fragment which encompasses the 2.9-kb *SstI* fragment of the c-Ha-ras-1 gene and additional flanking sequences both 5' and 3' to the coding sequence⁹; pEC, therefore, contains the potential enhancer element¹⁸. Table 2 shows that, after reaction with *anti*-BPDE and transfection, both plasmids induced transformed foci resulted at approximately equal efficiencies whether plasmid DNA in the form of supercoils and relaxed circles or DNA linearized with *EcoRI* was reacted with *anti*-BPDE (data not shown). The foci consisted of refractile, criss-crossing rounded cells, and many contained the giant cells typical of foci induced by tumour DNAs containing *ras* oncogenes. No foci were observed in any (0/25) of the plates transfected with untreated DNA. Additionally, no foci were observed when NIH 3T3 cells were transfected with pAT153, a plasmid that did not contain the c-Ha-ras-1 gene, but which had been reacted with *anti*-BPDE. Table 2 also shows that the numbers of foci induced varied with the ratio of DNA to *anti*-BPDE. The highest efficiency of focus production was observed for a ratio of 15:1 DNA/*anti*-BPDE. If more *anti*-BPDE was used, fewer foci were recovered, probably because the higher level of adducts was deleterious to the gene.

The levels of *anti*-BPDE-DNA adducts in aliquots of the reacted plasmids used for transfection were measured using ³²P-postlabelling analysis²⁰. At DNA/*anti*-BPDE ratios of ≥ 15:1, 4–6% of the carcinogen became covalently bound to DNA, the remainder being hydrated to non-reactive tetrols. Table 2 shows that at the highest level of focus formation (DNA/*anti*-BPDE = 15:1), an average of 1 nucleotide in 250 was modified. Assuming uniform distribution of adducts throughout the plasmid, at this level of modification the c-Ha-ras-1 coding sequence would contain an average of 2.3 adducts per DNA strand. The major DNA adduct was formed at the N² position of guanine^{21,22}. However, we also detected minor adducts that are thought to arise from reactions at other sites in guanine and from reactions with the exocyclic amino groups in adenine and cytosine residues²³.

To demonstrate that transformed foci contained the transfected genes, DNA was prepared from cultures of individual foci and subjected to Southern blot analysis, using probes specific for the c-Ha-ras-1 gene. Figure 1 shows that foci contained sequences derived from the transfected plasmids. It is apparent from the intensity of the 2.9-kb *SstI* fragment that, in many cases, multiple copies were present. Furthermore, multiple hybridizing bands were present when the cellular DNA was digested with *EcoRI*, an enzyme which cuts the plasmids only in vector sequences, showing that the primary transfectants contained plasmids integrated at several sites. It is likely that only some of the multiple copies are activated c-Ha-ras-1 genes, the remainder being non-transforming genes that were carried over in the transfection process. To simplify further analysis, therefore, we used DNAs from the primary transfectants in a second round of DNA transfection. As has been demonstrated many times, a second round of DNA transfection results in the loss of DNA sequences gained adventitiously in the first round. DNA from 23 of 26 primary foci transferred the transformed phenotype to NIH 3T3 cells, with an efficiency of 0.02–0.45 foci per µg DNA. These secondary transfers demonstrate that the transforming genes detected in the first round of transfection

Table 2 Induction of focus-forming activity following reaction of *anti*-BPDE with plasmids containing the c-Ha-ras-1 proto-oncogene

DNA/ <i>anti</i> -BPDE ratio	Total foci/total plates	pHa-1		pEC	
		Foci μg^{-1}	Fraction of nucleotides modified by BPDE	Total foci/total plates	Foci μg^{-1}
8:1	1/5	0.2	1/200	5/10	0.5
15:1	48/25	1.92	1/250	17/10	1.7
40:1	ND	—	—	13/10	1.3
80:1	12/20	0.6	1/1,250	4/10	0.4
300:1	3/25	0.12	1/3,350	0/10	0
800:1	1/10	0.1	1/11,600	ND	—
1:0	0/25	0	0	0/15	0

pHa-1 or pEC plasmid DNA was treated with *anti*-BPDE, precipitated and redissolved as described for Table 1. NIH 3T3 cells were treated with a calcium phosphate co-precipitate containing 0.2–1 μg plasmid DNA and 20 μg carrier mouse DNA. After transfection the medium was changed at 3–4-day intervals and foci scored after 16–18 days. Foci of transformed cells were isolated by trypsinization in small stainless steel cylinders. To determine the levels of *anti*-BPDE–DNA adducts, a sample (0.17 μg) of DNA that had been hydrolysed to deoxyribonucleoside 3'-monophosphates by micrococcal nuclease and spleen phosphodiesterase was incubated with [γ - ^{32}P]ATP (150 μCi) and T4 polynucleotide kinase (3.0 units) exactly as described elsewhere²⁰. The ^{32}P -labelled adducts were resolved from the labelled normal nucleotides by a four-directional anion-exchange polyethyleneimine–cellulose TLC system²⁰. The adduct spots were located by autoradiography, then assayed by Cerenkov scintillation counting. ND, not determined.

could be serially passaged. Southern blot analysis of these second round transfectants showed that they contained only single or double integration events, although the c-Ha-ras-1 genes were present in multiple copies in some instances (data not shown).

DNA sequence analysis of *ras* oncogenes cloned from human tumours has shown that activation results from point mutations in either the 12th or 61st codons^{8–11}. Point mutations in codons 11 or 12 of the c-Ha-ras-1 gene can be detected following digestion of the DNA with *Hpa*II or *Msp*I, as the last two bases of codon 11 and the first two of codon 12 form a CCGG recognition site⁸. Digestion of the normal c-Ha-ras-1 gene with *Msp*I and *Hpa*II and Southern blotting with a 600-base pair (bp) *Sma*I fragment of c-Ha-ras-1 results in fragments of 355 and 56 bp^{8,24}. Mutation at the CCGG site causes the loss of the recognition site, resulting in the production of a single larger fragment of 411 bp after digestion. Figure 2 shows that when DNAs from secondary foci were analysed on Southern blots after *Msp*I and *Hpa*II digestion, some of them (lanes d–g; j, k; n, o) contained a fragment with the mobility of the 355-bp fragment derived from the normal c-Ha-ras-1 gene (lane q), while others (lanes b, c; h, i; l, m) contained a larger fragment which co-migrated with the 411-bp fragment from the c-Ha-ras-1 oncogene present in EJ/T24 bladder carcinoma cells (lane p). The 56-bp fragment was not detected in the experimental conditions used here. As the EJ/T24 oncogene is known to have lost the CCGG site due to a GC $\rightarrow$ TA transversion in codon 12 (refs 8, 9), this result demonstrates that some of the transforming genes produced following reaction with *anti*-BPDE *in vitro* contain alterations in either codon 11 or 12. Of 17 independent foci derived from modified pEC analysed so far, 4 appear to have alterations in this region. Of four foci derived from pHa-1, one is altered at codon 11 or 12. Studies in *Escherichia coli* on mutation of the *lacI* gene have shown that *anti*-BPDE preferentially induces GC $\rightarrow$ TA transversions¹³. Sequence analysis of the transforming genes that we have produced will disclose the

nature of the mutations that occur in mammalian cells when DNA is modified by *anti*-BPDE. Whether the mutations reported here have arisen through erroneous replication of damaged DNA templates, or as a result of error-prone repair of the lesions, is unknown. However, from the design of our experiments it is clear that these mutations result from direct modification of the DNA rather than from misincorporation of modified bases into DNA, which has been demonstrated as a mechanism for mutagenesis by alkylating agents²⁵.

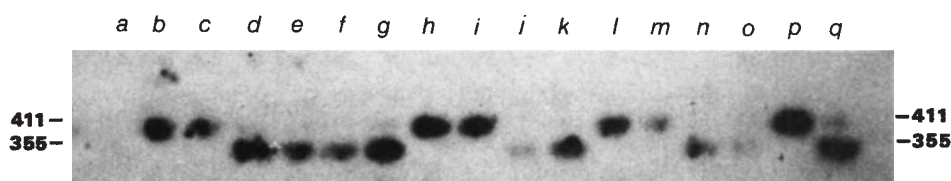
Several studies have shown that tumours which arise in animals following administration of chemical or other carcinogens contain activated *ras* oncogenes^{26–29}. The reproducible activation of *ras* oncogenes in these tumours suggests that the activation results from the action of the carcinogen on the proto-oncogene. However, because of the long delay (6–9 months) between administration of the carcinogen and appearance of the tumour, these studies do not show that the carcinogen is directly causing point mutations in *ras* proto-oncogenes. Our demonstration that mutations leading to transforming activity can be induced in a *ras* proto-oncogene by reaction with an ultimate carcinogen, therefore, shows that carcinogen binding can lead to mutation in proto-oncogenes and supports the concept that the induction of mutations following carcinogen–DNA binding is a necessary step in the multistage process of tumour induction.

The generation of transforming *ras* genes following mutation of a *ras* proto-oncogene is an unusual mutational system as it represents a forward mutation. This forward mutation is readily scored following transfection of the DNA into NIH 3T3 cells and the system should be useful for determining the mutational alterations produced by other mutagens and carcinogens. Preliminary results with other agents suggest that they are also capable of causing mutations in *ras* proto-oncogenes.

This paper is dedicated to the memory of Professor Peter Sims (1919–83) in recognition of his pioneering studies on polycyclic hydrocarbon activation carried out at this Institute.

Fig. 2 Southern blot showing presence and loss of the CCGG recognition site at codons 11 and 12 in DNA from secondary foci isolated after treating pEC with *anti*-BPDE. Lane a, NIH 3T3 DNA; lanes b and c, two secondary transfectants derived from primary focus 494; lanes d and e, two secondary transfectants derived from primary focus 495; lanes f and g, two secondary transfectants derived from primary 496; lanes h and i, two secondary transfectants derived from primary 497; lanes j and k, two secondary transfectants derived from primary 501; lanes l and m, two secondary transfectants derived from primary 505; lanes n and o, two secondary transfectants derived from primary 508; lane p, pEC; lane q, pEC.

Methods: 20 μg of DNA were digested to completion with *Msp*I and *Hpa*II, electrophoresed in a 3% agarose gel and transferred to a nitrocellulose filter. The filter was hybridized with 8×10^7 c.p.m. of nick-translated ^{32}P -labelled probe of the 600-bp *Sma*I fragment from pHa-1 using conditions described elsewhere²⁴. The filter was washed in $0.1 \times \text{SSC}$ 0.1% SDS at 60 °C and autoradiography was carried out at –70 °C for 72 h. In these conditions no cross-hybridization of the probe to the endogenous NIH 3T3 c-Ha-ras sequences was detected.



We thank Drs R. A. Weinberg and E. Chang for samples of pEJ, pEC and pHa-1; M. Glennon and P. M. Jones for technical assistance; and Dr A. Hall for helpful discussions. This work was jointly supported by the C.R.C. and the MRC.

Received 3 April; accepted 4 June 1984.

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Direct evidence that oncogenic tyrosine kinases and cyclic AMP-dependent protein kinase have homologous ATP-binding sites

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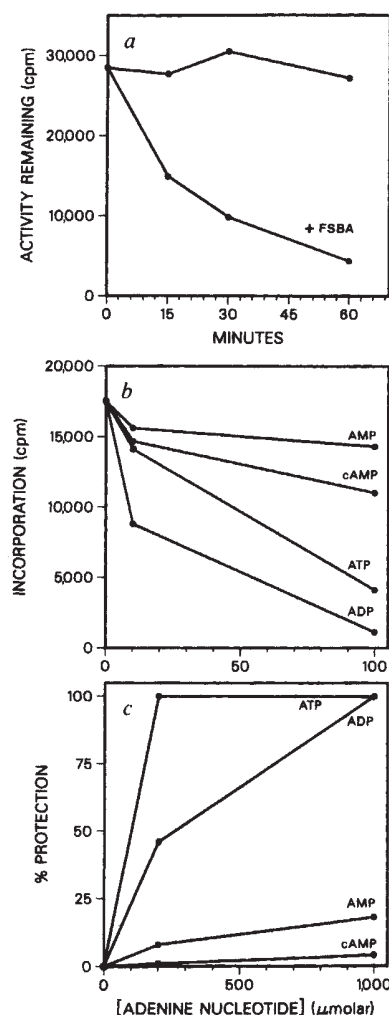
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p60^{src}, the transforming protein of Rous sarcoma virus¹ (RSV), is a protein kinase² that has a strict specificity for tyrosine³. The phosphorylation of cellular proteins by p60^{src} (ref. 4) results in transformation. Recently, Barker and Dayhoff⁵ discovered that residues 259-485 of p60^{src} have 22% sequence identity with residues 33-258 of the catalytic subunit of cyclic AMP-dependent protein kinase, an enzyme that has a specificity for serine. Because it was necessary to introduce eight gaps to align the two proteins, the question remained as to whether this apparent homology reflected a common evolutionary origin. We demonstrate here that the ATP analogue *p*-fluorosulphonylbenzoyl 5'-adenosine (FSBA) inactivates the tyrosine protein kinase activity of p60^{src} by reacting with lysine 295. When aligned for maximum sequence identity, lysine 295 of p60^{src} and the lysine in the catalytic subunit which also reacts specifically with FSBA are superimposed precisely. This functional homology is strong evidence that the protein kinases, irrespective of amino acid substrate specificity, comprise a single divergent gene family.

Fig. 1 *a*, FSBA inactivates the phosphotransferase activity of p60^{src}; *b*, competition by adenine nucleotides; *c*, protection by adenine nucleotides. Each point in *b* and *c* represents the average of two determinations.

Methods: *a*, SR-RSV-A-infected chicken cells were lysed in Nomidet P-40 (NP40) buffer (150 mM NaCl, 10 mM sodium phosphate pH 7.2, 1% NP40, 1% Trasylol, 2 mM EDTA) and p60^{src} was isolated by immunoprecipitation with rabbit anti-carboxy-terminal peptide serum⁸. The immunoprecipitate was washed in the same buffer and aliquots were frozen at -80 °C. For each experiment in *a*-*c*, a single immunoprecipitate was thawed and washed once in phosphate-buffered saline (PBS; 10 mM sodium phosphate pH 7.2, 150 mM NaCl). In *a*, half was incubated at 30 °C for 0, 15, 30 or 60 min in kinase buffer alone (10 mM sodium phosphate pH 7.2, 5 mM MgCl₂, 1.3% dimethyl sulfoxide) and the other half in kinase buffer with 1.0 mM FSBA. At each time point an aliquot was removed, washed twice with 150 mM NaCl, 10 mM sodium phosphate pH 7.2, 1% NP40, once in kinase buffer and stored as a pellet on ice. When all the preincubations had been completed, the samples were resuspended in 5 µl of 10 mM sodium phosphate pH 7.2, 5 mM MgCl₂, 5 µCi ³²P-ATP (3,000 Ci mmol⁻¹; Amersham), 1.0 µM cold ATP, 2 mM [Val⁵]angiotensin II (Sigma) and incubated at 30 °C for 4 min. These conditions produced linear incorporation of ³²P into [Val⁵]angiotensin II throughout the reaction. p60^{src} was inactivated by heating the sample to 90 °C for 1 min. All samples were analysed by one-dimensional electrophoresis at pH 3.5 on cellulose thin-layer plates as described elsewhere⁹. *b*, A single p60^{src} immunoprecipitate was washed once in PBS and equivalent aliquots were allowed to phosphorylate [Val⁵]angiotensin II for 4 min in the presence of 10 µM or 100 µM competing adenine nucleotide as described above. The concentration of ³²P-ATP, 1.3 µM, was below the K_m for ATP of p60^{src} which is 12 µM³⁰. This explains the apparent lack of competition at 10 µM ATP. Competition is pronounced, however, at 100 µM ATP, which is well above the K_m. *c*, A single immunoprecipitate of p60^{src} was washed once in PBS and equivalent aliquots were preincubated for 45 min in kinase buffer and 1.0 mM FSBA in the presence of the indicated adenine nucleotide at a concentration of either 200 or 1,000 µM. After 45 min, the slurry was washed twice with kinase buffer supplemented with 1% NP40 and once in kinase buffer alone. Remaining kinase activity was determined by the assay described for *a*.

The structure of FSBA is similar to that of ATP except that the three phosphates have been replaced by a side chain of similar size, which contains a reactive sulphonyl fluoride group in the position of the γ-phosphate^{6,7}. Using an antiserum specific for the carboxy terminus of p60^{src} (ref. 8), we isolated the enzyme by immunoprecipitation in a form capable of phosphorylating [Val⁵]angiotensin II (ref. 9) with linear kinetics. Preincubation with 1.0 mM FSBA destroyed the phosphotransferase activity of p60^{src} in a time-dependent fashion (Fig. 1*a*). If FSBA inactivates p60^{src} by reaction with the ATP-binding site, the strength of various adenine nucleotides as competitive inhibitors of p60^{src}



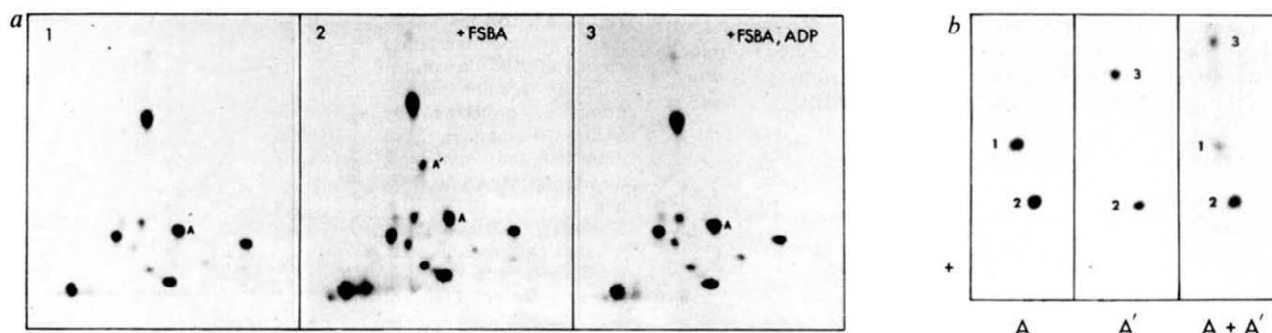


Fig. 2 *a*, $p60^{src}$ incubated with FSBA yields a novel tryptic peptide. 1, $p60^{src}$; 2, $p60^{src}$ incubated with 200 μ M FSBA; 3, $p60^{src}$ incubated with 200 μ M FSBA and 1.0 mM ADP. Tryptic peptides A and A' are indicated. *b*, Tryptic peptides A and A' are related. A, *S. aureus* protease digest of tryptic peptide A; A', *S. aureus* protease digest of tryptic peptide A'. A + A', mixture of *S. aureus* protease digests of peptides A and A'.

Methods: *a*, Three immunoprecipitates containing $p60^{src}$ were prepared with rabbit anti-RSV tumour serum from three 100-mm dishes of PR-RSV-C, stock 1^{31} infected chicken cells which had been labelled overnight in Dulbecco's minimal essential medium containing 10% of the normal concentration of methionine, 4% dialysed calf serum and 1.0 mCi 35 S-methionine (1,400 Ci mmol $^{-1}$; Amersham). Cells were lysed in NP40 buffer (see Fig. 1) and immunoprecipitates washed in the same buffer. One immunoprecipitate was incubated at 30 °C in kinase buffer alone, a second in kinase buffer supplemented with 200 μ M FSBA and the third with kinase buffer supplemented with both 200 μ M FSBA and 1.0 mM ADP. After incubation for 40 min, the immunoprecipitates were each washed three times in 150 mM NaCl, 10 mM sodium phosphate pH 7.2, 0.1% NP40 and boiled in sample buffer supplemented with 50 mM Tris-HCl pH 6.8, to destroy the sulphonyl fluoride groups of residual FSBA molecules. Samples were fractionated on SDS-polyacrylamide gels which were dried without fixation. $p60^{src}$ was located by autoradiography, eluted, oxidized with performic acid, subjected to exhaustive digestion with trypsin and analysed by two-dimensional mapping as described previously^{32,33}. Electrophoresis on cellulose TLC plates was carried out at 1.0 kV for 27 min at pH 4.72. Electrophoresis was from left to right with the origin on the left and the negative electrode on the right. Ascending chromatography was performed for 6 h in a buffer composed of butanol/pyridine/acetic acid/H₂O, 97:75:15:60 (v/v), pH 5.3. In this buffer hydrophobic peptides migrate more rapidly than hydrophilic ones. Plates were dried after chromatography, dipped in 2-methylnaphthalene, 0.4% diphenyloxazole, and the peptides visualized by fluorography. *b*, Peptides A and A' were isolated by elution from two-dimensional maps (see Fig. 3) and digested exhaustively with *S. aureus* V8 protease as described previously³⁴. The digestion products were analysed by mapping as in Fig. 3, except that electrophoresis was for 8 min instead of 27 min.

should be proportional to their ability to protect the ATP-binding site from reaction with FSBA. ADP and ATP were potent competitive inhibitors of $p60^{src}$ when assayed using [Val⁵]angiotensin II as substrate. Both were also very effective in protecting $p60^{src}$ from inactivation by FSBA (Fig. 1*b, c*). AMP and cyclic AMP, however, were both poor competitive inhibitors and ineffective in protecting the enzyme from inactivation by FSBA. We conclude that FSBA inactivates $p60^{src}$ by reacting with the region that binds ATP.

Inspection of a two-dimensional map of the 35 S-methionine-labelled tryptic peptides of $p60^{src}$ from Prague RSV of subgroup C (PR-RSV-C) reveals that modification of $p60^{src}$ by FSBA caused a single methionine-containing peptide, designated A, to decrease in intensity and a new peptide, designated A' to appear (Fig. 2*a*). Secondary digestion of these two peptides with *Staphylococcus aureus* V8 protease¹⁰ produced one identical peptide and one unique peptide (Fig. 2*b*). A and A' were therefore related. The disappearance of A and appearance of A' were both prevented by the presence of a fivefold excess of Mg²⁺-ADP (Fig. 2*a*). Because Mg²⁺-ADP blocks both enzymatic inactivation by FSBA and the covalent modification responsible for the appearance of peptide A', we reasoned that an amino acid within peptide A' is located within the ATP-binding site of $p60^{src}$.

To determine which amino acid in peptide A' had reacted with FSBA, peptides A and A' were isolated and subjected to automated amino-terminal sequence analysis. Methionine residues were found at positions 7 and 19 in peptide A and at positions 11 and 23 in peptide A' (Fig. 3). Peptide A' therefore differs from peptide A in that it contains four additional amino acids on its amino-terminal end. This would occur if FSBA modified a site of tryptic cleavage, rendering it insensitive to digestion by trypsin and fusing peptide A with an adjacent tryptic peptide composed of four amino acids. Inspection of the sequence of $p60^{src}$ reveals that only the tryptic peptide containing residues 292–315 could be peptide A'. Peptide A would therefore contain residues 296–315. Consequently, FSBA modified lysine 295 (Fig. 3).

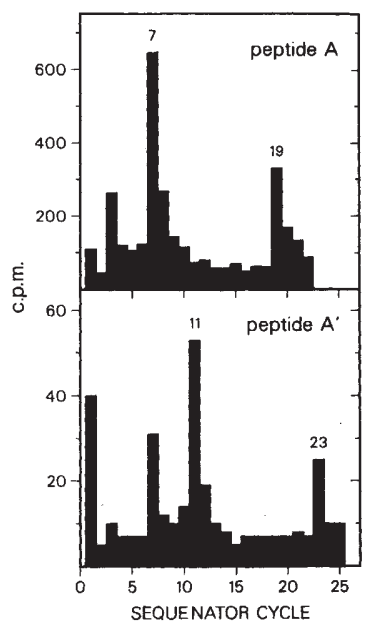
FSBA is known to react specifically with the ATP-binding

site of cyclic AMP-dependent protein kinase and to modify lysine 71¹¹. In addition, FSBA reacts with a homologous lysine residue in the cyclic GMP-dependent protein kinase, which has 42% sequence homology with the cyclic AMP-dependent protein kinase within this region¹². Lysine 295 of $p60^{src}$ aligns precisely with the reactive lysines found in these cyclic nucleotide-dependent protein kinases. Therefore, the tertiary structures of the ATP-binding regions of both cyclic nucleotide-dependent serine kinases and the tyrosine kinase $p60^{src}$ all position a homologous lysine residue such that it reacts with FSBA. This is compelling evidence that the sequence homology exhibited by $p60^{src}$ and these serine kinases reflects structural and functional homology, and reinforces the notion that the protein kinases, irrespective of their amino acid substrate specificity, share a common ancestry.

In addition to the *src* gene of RSV, six other classes of RNA tumour viruses harbour transforming genes which encode or are likely to encode tyrosine protein kinases. The sequence of five of these genes, *yes* (ref. 13), *fps* (ref. 14), *abl* (ref. 15), *fgr* (ref. 16) and *erbB* (ref. 17) (whose cellular homologue encodes the epidermal growth factor receptor^{18,19}), are known. They exhibit 90, 48, 51, 82 and 38% amino acid sequence homology, respectively, with the catalytic domain of $p60^{src}$ (ref. 20). Each contains a lysine residue at a position homologous to lysine 295 of $p60^{src}$ (Fig. 4).

In all these proteins, a cluster of glycines with the sequence Gly-X-Gly-X-X-Gly lies 16–28 residues to the amino-terminal side of the lysines implicated in binding ATP (Fig. 4). X-ray crystallographic studies have shown that a region containing an identical constellation of glycines can be found between two β -pleated sheets that form the adenine dinucleotide binding pocket of lactate dehydrogenase (LDH)²¹. This region functions as an essential 'elbow' adjacent to the ribose-phosphate moiety of the adenine dinucleotide portion of NAD^{21,22} and is proposed to perform the same function in the GTP-binding domain of p21^{ras} (ref. 23), the transforming protein of Harvey and Kirsten sarcoma viruses. Alcohol dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase and glutathione reductase also bind adenine nucleotides as cofactors²¹, but have less than 25%

Fig. 3 Sequence analysis of tryptic peptides A and A'. Peptides A and A' were isolated by aspiration from two-dimensional maps (see Fig. 2) and eluted three times with a total of 600 μ l of pH 4.72 electrophoresis buffer. These eluates were pooled, lyophilized and dissolved in 0.2 ml of 0.1 M NaHCO₃ (pH 9.0) containing 10% n-propanol. 50 mg of aminoethylamino propyl PG-500-200 Controlled Pore glass beads (Sigma) that had been activated with phenyl diisothiocyanate³⁵ were then added. After coupling was complete, the immobilized peptide was subjected to sequence analysis by the Edman degradation procedure³⁶ using a solid-phase peptide sequencer³⁷. The graphs present the radioactivity recovered at each cycle. The predicted amino acid sequence encompassing peptides A and A' is shown at the bottom. Arrows indicate tryptic cleavage sites. The predicted sequences of tryptic peptide A and the FSBA-modified tryptic peptide A' are also shown. The major peaks of radioactivity correlate with the positions of methionine residues. The radioactivity found in cycle 3 in the analysis of tryptic peptide A and in cycle 7 in the analysis of tryptic peptide A' is unlikely to be due to methionine residues at these positions. Lysine 299 is resistant to tryptic cleavage because it is followed by a proline. Some fraction of the peptides will be coupled to the solid support through both their α -amino group and the ϵ -amino group of lysine 299 but not through their carboxy-terminal lysine 315. These peptides will be released from the resin at step 3 (peptide A) or at step 7 (peptide A'). To obtain highly radioactive quantities of tryptic peptide A for analysis, we found it convenient to isolate p60^{src} from bacteria containing a PR-RSV-A *lac*-*src* expression vector³⁸ rather than from infected cells. 1 ml of exponentially growing bacteria, at an A₅₅₀ of 0.6, were incubated for 10 min at 37 °C in methionine-free minimal medium. Transcription of the *lac* promoter was induced with 1.0 mM isopropyl thioalactoside for 2 min, and the cells were then labelled with 0.5 mCi ³⁵S-methionine for 20 min in methionine-free medium. p60^{src} was isolated by immunoprecipitation from the bacterial lysate in RIPA buffer using a rabbit anti-RSV tumour serum³⁹.



sequence homology with LDH. All contain this cluster of glycines. The Gly-X-Gly-X-X-Gly region of the protein kinases may also have a similar role in binding ATP.

The proteins encoded by the *mos* (ref. 24), *mil/raf* (refs 25, 41) and *fms* (ref. 26) viral oncogenes also have homology with the kinase domain of p60^{src}. All their products, however, lack obvious kinase activity²⁷⁻²⁹. The fact that all encode a lysine residue in a position homologous to that of lysine 295 of p60^{src} and that each contains a similar cluster of glycines (Fig. 4) implies that each may well have nucleotide-binding activity.

We argue that it is significant that the residue with which FSBA reacts is lysine. The positive charge carried by the ϵ -amino group of this lysine could withdraw electron density from the γ -phosphate of ATP and activate it for nucleophilic attack by the -OH group of a serine, threonine or tyrosine residue. Alternatively, this lysine could act as a general base by abstracting a proton from the hydroxyl group of the amino acid substrate. We predict that alteration of lysine 295 in p60^{src}, or of the homologous lysines in the other viral tyrosine protein kinases, by site-directed mutagenesis of cloned DNA, will eliminate both kinase activity and transforming activity.

We thank Tony Hunter for encouragement and for comments on the manuscript. This work was supported by USPHS grants CA-14195, CA-17289 and GM-19301.

Received 25 April; accepted 12 June 1984.

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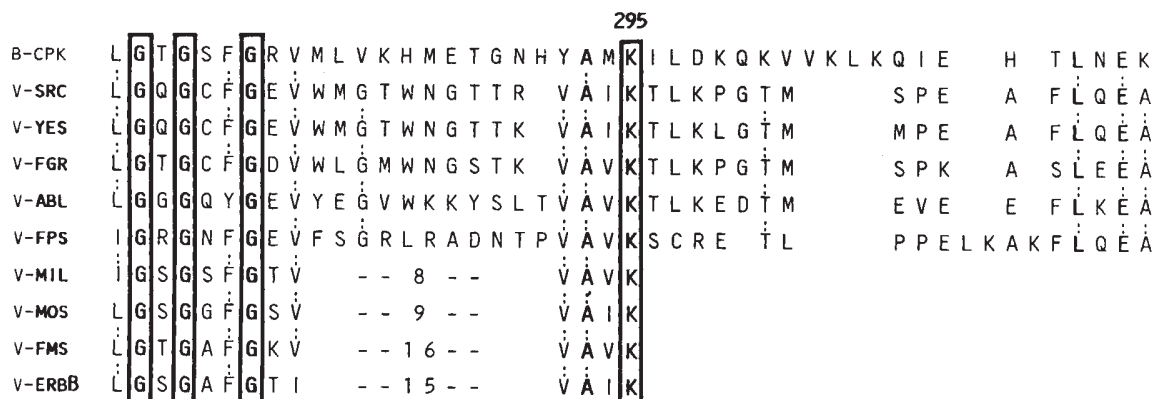


Fig. 4 Alignment of amino acid sequences surrounding one ATP-binding region of the kinase family. Amino acids homologous to lysine 295 and the Gly-X-Gly-X-X-Gly sequence of p60^{src} are boxed. The sequences presented correspond to residues 38-132 of the bovine catalytic subunit⁴⁰, 273-351 of p60^{src} (ref. 20), 544-769 of p90^{src-yes} (ref. 13), 927-1,009 of p140^{src-fps} (ref. 14), 368-448 of p120^{src-abl} (ref. 15), 273-295 of p67^{src-bb} (ref. 17), 100-181 of p37^{src} (ref. 24), 602-622 of p100^{src-mil/raf} (ref. 41) and 463-491 of p160^{src-fms} (ref. 26). Amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

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Expression of *c-myc* changes during differentiation of mouse erythroleukaemia cells

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The transforming gene of avian myelocytomatosis virus MC29, *v-myc*, causes a variety of malignancies in chickens¹. A cellular homologue², *c-myc*, has been implicated in B-cell malignancies in mice and humans³⁻⁶ but is also expressed in many normal cell types⁷ and may be important in the control of normal cell proliferation⁸. *c-myc* is highly conserved in vertebrates⁹. We have been investigating the relationship between *c-myc* expression and the terminal differentiation of cultured mouse erythroleukaemia (MEL) cells. We find that the level of *c-myc* messenger RNA shows a rapid biphasic change in MEL cells induced to differentiate by dimethyl sulphoxide or hypoxanthine. The changes occur during the first few hours of the differentiation programme and require active protein synthesis. These data suggest that changes in *c-myc* expression may be important in the irreversible commitment of MEL cells to terminal erythroid differentiation.

MEL cells are transformed erythroid precursor lines that can be induced to differentiate into mature erythroid cells by a variety of chemicals^{10,11}. Although continuous exposure to the inducer is not required for differentiation to occur, a latent period of 12-24 h is necessary before an increase in the number of committed cells, capable of differentiating in the absence of inducer, can be detected^{11,12}. After 48-72 h exposure to 1.8% dimethyl sulphoxide (DMSO) 80-90% of MEL cells undergo irreversible commitment.

Logarithmic cultures of MEL cells (line 745) were induced with 1.8% DMSO. Aliquots of cells were removed before DMSO treatment and at various time points afterwards. Total cellular RNA was extracted¹³ and analysed on RNA blots after electrophoresis in denaturing agarose gels¹⁴. *c-myc* expression was analysed by hybridizing the blots with pc-myc-Bam/Bam^{15,16}, a recombinant plasmid containing a 5.5 kilobase (kb) *Bam*HI fragment of a rearranged mouse *c-myc* gene which includes exons 2 and 3. Untreated MEL cells contain readily detectable amounts of a 2.3-kb transcript that hybridizes with the *c-myc* probe (Fig. 1a, lane 1). This transcript is the same size as the *c-myc* mRNA found in normal mouse spleen¹⁷ and lymphoid cell lines¹⁸. After 2 h of DMSO treatment, the level of *c-myc* mRNA decreased approximately 15-fold. *c-myc* RNA remained low until 12 h but increased to pretreatment levels by 18 h (Fig. 1a). Thereafter *c-myc* mRNA declined gradually, as terminally differentiated benzidine-positive cells accumulated after 24-120 h of DMSO treatment¹¹ (data after 48 h not shown). The early biphasic change in *c-myc* mRNA levels seen in Fig. 1a was observed in four separate DMSO induction experiments. In some experiments the initial drop in *c-myc* mRNA occurred as early as 1 h after DMSO treatment (Fig. 3a).

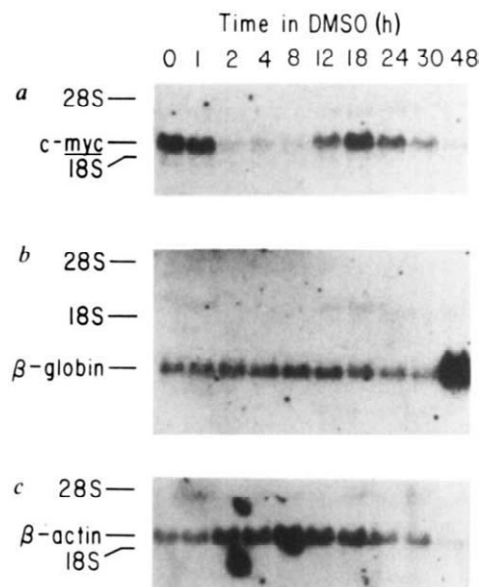


Fig. 1 Relative levels of *c-myc*, β -globin and β -actin mRNAs during DMSO induction of MEL cells. *a*, RNA blot hybridization with ³²P-labelled pc-myc-Bam/Bam, described in the text. Autoradiographic exposure time was 72 h. Densitometric analysis of the autoradiogram using a Joyce Loebl densitometer indicated that the level of hybridization to *c-myc* mRNA at 2, 4 and 8 h was less than 7% of the level at 0 h. *b*, The blot in *a* was incubated at 80 °C for 3 h in 0.1 × Denhardt's solution, 5 mM Tris-HCl, pH 8.0, 0.2 mM Na₂-EDTA and 0.05% sodium pyrophosphate to remove hybridized radioactivity. It was then hybridized with a mouse β -globin cDNA recombinant, pCR1 β MG9 (ref. 20). Autoradiographic exposure time was 20 h. *c*, The blot in *b* was treated as above to remove hybridized radioactivity and then hybridized with a chicken β -actin cDNA recombinant, pAC269 (ref. 21). Autoradiographic exposure time was 5 h. RNA was prepared from a single logarithmic culture of MEL cells, induced by diluting the culture to 2 × 10⁵ cells ml⁻¹ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U ml⁻¹ penicillin-streptomycin and 1.8% DMSO. Aliquots of cells were removed at the indicated times. The proportion of benzidine-positive cells in the culture, determined as previously described²⁸, was: 0-18 h, 1%; 24 h, 3%; 30 h, 8%; 40 h, 28%; 120 h, 80%. Total RNA was prepared by phenol extraction at 60 °C^{13,14}. RNA from 2 × 10⁶ cells was fractionated by electrophoresis and transferred to nitrocellulose paper¹⁴. Hybridization probes were labelled to specific activities of 0.5-1 × 10⁸ d.p.m. μ m⁻¹ by nick-translation with ³²P-TTP and dCTP (ref. 29). The blots were incubated at 65 °C for 4 h in a solution containing 5 × SSC, 10 × Denhardt's solution, 50 mM sodium phosphate, pH 6.8 and 50 μ g ml⁻¹ denatured salmon sperm DNA and then hybridized at 65 °C for 16 h in the same solution containing 2 × 10⁶-5 × 10⁶ d.p.m. ml⁻¹ of the specific probe. Unhybridized radioactivity was removed by incubation at room temperature for 30 min in 2 × SSC and 0.1% SDS, followed by incubation at 55 °C for 1 h in 0.1 × SSC and 0.1% SDS. The blots were dried and exposed to Kodak XAR-5 film with two intensifying screens. The positions of 28S and 18S ribosomal RNAs in the gel, visualized by ethidium bromide staining, are indicated in the figure.

A similar biphasic change in *c-myc* mRNA levels was observed on treatment of cells with hypoxanthine, another inducer of MEL cell differentiation¹⁹ that is unrelated in chemical structure to DMSO (Fig. 2). The biphasic change in *c-myc* mRNA levels observed after hypoxanthine treatment differed from that seen in cells treated with DMSO, in that the restoration of *c-myc* mRNA level occurred earlier (at 4 h) and was more pronounced. Southern blot analysis of restriction enzyme digested genomic DNA from MEL cells did not reveal amplification or gross rearrangement of the *c-myc* gene (data not shown).

To compare the changes in *c-myc* mRNA levels resulting from DMSO treatment with that of other mRNAs, the blot shown in Fig. 1a was erased and rehybridized with two other mRNA-specific probes. Figure 1b shows the results obtained by hybridiz-

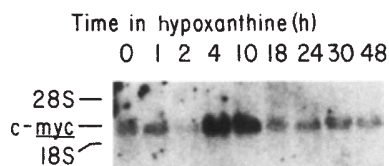


Fig. 2 Changes in *c-myc* mRNA content during MEL cell induction with hypoxanthine. Cell culture was performed as described in Fig. 1 except that induction was carried out in medium supplemented with 5 mM hypoxanthine. Procedures for RNA preparation and analysis were as described in Fig. 1 legend except that 20 μ g of total RNA was used in each lane. The proportion of benzidine-positive cells in the culture at the indicated times was: 0–24 h, 1–2%; 30 h, 4%; 48 h, 12%; 120 h, 60%. Autoradiographic exposure time was 48 h.

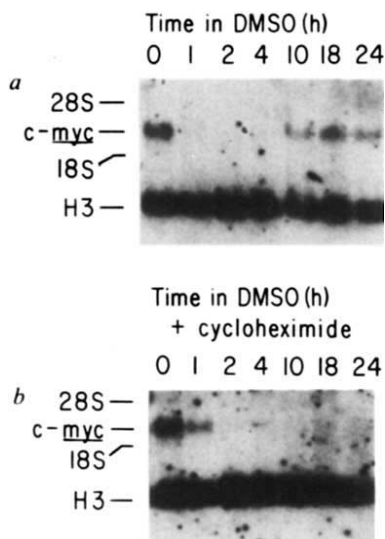


Fig. 3 Effect of protein synthesis inhibition on the changes in *c-myc* mRNA levels in DMSO-treated MEL cells. *a*, MEL cells incubated at 37 °C in the presence of 1.8% DMSO. *b*, Cells incubated as *a* with the addition of 0.5 μ g ml⁻¹ cycloheximide, a dose that has been shown to inhibit 90% of protein synthesis and to inhibit reversibly cell division and commitment of MEL cells³⁰. A logarithmic culture of MEL cells was diluted to 3×10^5 cells ml⁻¹ in growth medium and then split into two. The cultures were incubated at 37 °C for 5 h in the presence or absence of cycloheximide. At time 0 both cultures were supplemented with 1.8% DMSO and incubation was continued at 37 °C for 24 h. Procedures for RNA preparation and analysis were as described in Fig. 1. The blots were hybridized simultaneously with ³²P-labelled pc-myc-Bam/Bam and a mouse histone H3 recombinant pRAH3-2 (ref. 22). Autoradiographic exposure time was 48 h. An exposure time of 24 h showed that H3 mRNA levels were relatively constant during the course of the experiment.

ation with a mouse β -globin cDNA recombinant, pCR1 β MG9 (ref. 20). Untreated cultures of MEL cells contain a low level of β -globin mRNA¹¹. In contrast to the rapid decline and subsequent increase seen for *c-myc* mRNA, the basal level of β -globin mRNA was relatively constant during the first 30 h of DMSO treatment. By 48 h, a large increase in the level of β -globin mRNA was seen, correlating with the presence of 28% benzidine-positive cells observed in the culture at this time. β -actin mRNA levels were analysed by hybridization with a chicken actin cDNA clone, pAC269 (ref. 21), which cross-hybridizes to mouse β -actin mRNA. The level of the 2.2-kb β -actin mRNA (Fig. 1c) increased moderately during the first 18 h of DMSO treatment. Thereafter the level declined gradually as the cells underwent terminal differentiation. Finally, in a

separate induction experiment, we also determined the levels of histone H3 mRNA by hybridization with a mouse H3 gene probe, pRAH3-2 (ref. 22). H3 mRNA levels were nearly constant during the 24 h of observation in this DMSO induction experiment (Fig. 3a).

To determine whether changes in *c-myc* expression induced by DMSO in MEL cells requires protein synthesis, we studied the effects of cycloheximide treatment on *c-myc* mRNA levels. Figure 3 compares the results from cells exposed to DMSO alone (Fig. 3a) with those from cells exposed to DMSO plus cycloheximide (Fig. 3b). Two differences in the pattern are noted. First, whereas the DMSO-induced decline occurs in the presence of cycloheximide, the decline is delayed by about 1 h. Second, the restoration of *c-myc* mRNA levels which occurs in DMSO-treated cells at about 12 h, does not occur in the presence of cycloheximide and DMSO even after 24 h of treatment. The level of H3 histone mRNA was also assayed in these samples (Fig. 3a, b) and was found to be relatively constant during the 24 h treatment with DMSO, with or without cycloheximide. We conclude that the decline and re-expression of *c-myc* mRNA levels have different requirements for protein synthesis. The DMSO-induced decline does not absolutely require new protein synthesis, although the rate of decline is reduced in the absence of protein synthesis. In contrast, the reappearance of *c-myc* mRNA is dependent on continued protein synthesis.

The results reported here show that *c-myc* mRNA levels in MEL cells undergo very rapid dramatic changes during the first 12 h of chemically induced differentiation. The fact that these changes are specific for *c-myc* mRNA and occur during the latent period of induction, before irreversible commitment takes place, suggests that they may be important events in preparing the cells for terminal differentiation.

c-myc mRNA levels were also observed to decline gradually during the later stages of differentiation. A reduction in *c-myc* mRNA has been noted after terminal differentiation in the human promyelocytic line HL-60 (ref. 23) and F9 teratocarcinoma cells²⁴. The early changes, however, are specific for *c-myc* RNA, while both β -actin (Fig. 1c) and histone H3 mRNAs (our unpublished observations) also decline in MEL cells during this period. These late changes, therefore, probably indicate a more general restriction in gene expression which reflects nuclear and chromatin condensation during terminal differentiation of MEL cells.

It is possible that the early changes in *c-myc* expression could be secondary to other changes, for example the distribution of cells in the mitotic cell cycle. *c-myc* is thought to be expressed in G₁ phase of the cell cycle²⁵ and DMSO induction of MEL cells has been reported to lead to a transient accumulation of cells in G₁ after ~12 h^{26,27}. However, we believe such an explanation to be unlikely for two reasons. First, hypoxanthine does not lead to accumulation of cells in G₁ (ref. 27) and yet this inducer caused a very rapid biphasic change in *c-myc* mRNA levels. Second, the level of histone H3 mRNA, which is markedly reduced in G₁ and G₂ MEL cells²², was found to be constant during the first 24 h of DMSO treatment. If cell cycle-related events were responsible for changes in *c-myc* mRNA content during DMSO treatment, we would have expected to observe changes in the level of H3 mRNA.

The mechanisms regulating *c-myc* expression are not known. Recent studies of *c-myc* expression in mitogen-stimulated cells indicate that mitogenic induction of *c-myc* mRNA does not require new protein synthesis. Inhibition of protein synthesis during mitogenic stimulation leads to 'superinduction' of *c-myc* mRNA, suggesting that *c-myc* mRNA levels may be regulated by a labile, protein inhibitor²⁵. The observation that the early decline in *c-myc* mRNA in DMSO-induced MEL cells is delayed by protein synthesis inhibition is consistent with such a model. However, the fact that the decline occurs in the presence of cycloheximide suggests that if a negative regulator is present, it is not labile in these cells. In contrast to mitogenic induction of *c-myc* mRNA, the restoration of *c-myc* mRNA levels in MEL cells 12–18 h after DMSO treatment requires the continuation

of protein synthesis. This suggests that *c-myc* gene expression is also controlled by a positively acting protein factor.

We thank Dr M. Scharff for pc-myc-Bam/Bam; Dr R. Hickey for pAC269; Dr R. B. Alterman for pRAH 3-2 and Alice Piranio for secretarial assistance. This work was supported by NIH grants PHS CA 16368 and 13330. A.S. is a recipient of an Irma T. Hirsch Career Scientist Award. H.M.L. is a recipient of a Cooley's Anemia Foundation Fellowship.

Received 20 April; accepted 4 July 1984.

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Cell type-specific enhancer element associated with a mouse MHC gene, E_β

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Class II molecules of the major histocompatibility complex (MHC) are heterodimeric glycoproteins expressed on the surface of antigen-presenting B lymphocytes and macrophages¹. The genes encoding the α - and β -chains of the class II heterodimers, $A_\alpha A_\beta$ and $E_\alpha E_\beta$, have recently been characterized at the molecular level^{2–5}, and certain cloned genes were shown to be functionally expressed after introduction into cells by DNA-mediated gene transfer^{6,7}. One study⁷ found that a transfected E_β gene was expressed in a macrophage cell only after treatment of cells with γ -interferon. DNA sequences associated with transfected Class II MHC genes may therefore have a regulatory role in their cell type-specific expression. We report here the identification of a cell type-specific transcriptional enhancer element associated with the mouse E_β gene.

We⁸ and others^{9,10} have recently shown that the tissue-specific expression of immunoglobulin heavy-chain genes is regulated by an immunoglobulin heavy-chain gene-associated enhancer element located between J_H and C_μ . This enhancer functions in cells which normally express heavy-chain genes but not in cells derived from other tissues, for example, fibroblasts. The possibility that an enhancer element might be associated with the mouse E_β gene was tested by subcloning restriction fragments of the gene and its flanking regions into the *EcoRI* site of plasmid pSER and testing the ability of the recombinant plasmids to transform the B lymphoma line, A20-2J (ref. 11) to the gpt^+ phenotype (growth in the presence of mycophenolic acid). This plasmid was derived from plasmid pSV2gpt (ref. 12) by removing the simian virus 40 (SV40) enhancer sequence⁸. In the absence of any E_β gene fragments, plasmid pSER transformed A20-2J cells to gpt^+ at a frequency of $<5 \times 10^{-6}$ while the transformation frequency obtained with the parental plasmid, pSV2gpt (containing the SV40 enhancer) was $\sim 10^{-4}$. We conclude from these data and similar results with the myeloma cell line, J558L (ref. 8), that plasmid pSER is dependent on the addition of enhancer sequences for efficient transformation.

Only one region out of 28 kilobases (kb) of E_β coding and flanking sequences (Fig. 1) was found to be positive in this assay—a 4.1-kb *HindIII*–*EcoRI* fragment containing the first exon and approximately 2.7 kb of upstream sequence (see Table

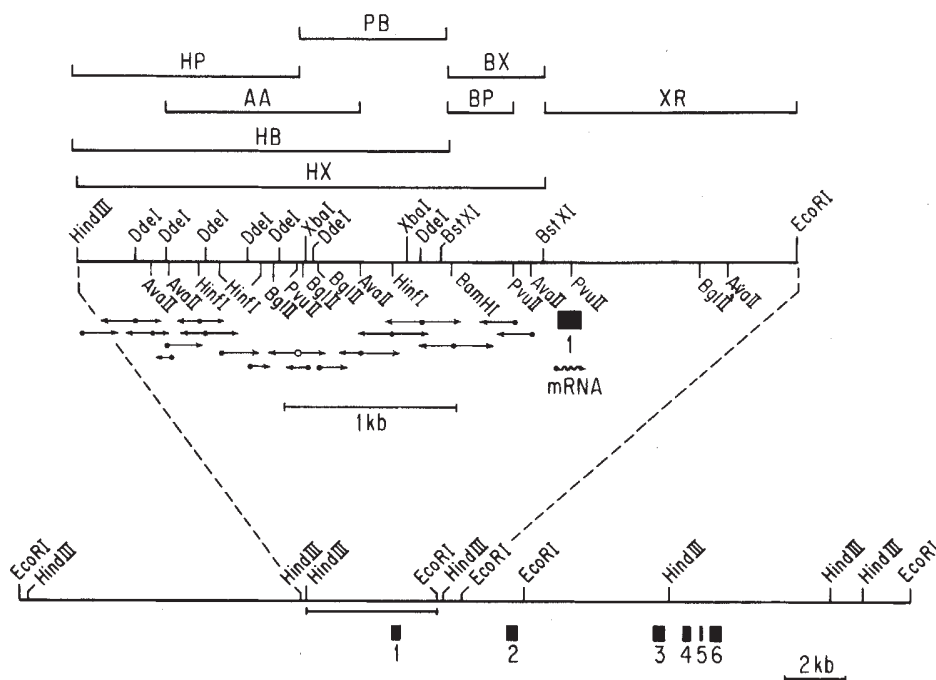


Fig. 1 Restriction map of the mouse E_β gene and flanking regions. The locations of the coding exons (solid boxes) are from ref. 5. The direction of transcription is from left to right. The 4.1-kb *HindIII* to *EcoRI* site is enlarged and the sequencing strategy of the 5' flanking region is indicated with arrows. Subfragments used to test enhancing activity (Table 1) are shown above and are abbreviated by letters which correspond to their 5' and 3' restriction sites.

1a). As the site of insertion of this fragment in plasmid pSER is some distance (2.5 kb) away from the SV40 promoter, and the 4.1-kb fragment worked equally well in both orientations (see below), we tentatively conclude that an enhancer element is located in this region of the E_{β} gene.

The subfragments shown in Fig. 1 were then tested for their ability to substitute for the SV40 enhancer. These fragments included three which extend from the 5' end of the 4.1-kb fragment (*Hind*III site) but differ at their 3' ends. As seen in Table 1b, fragments extending either to the *Bst*XI site near the E_{β} gene promoter (HX fragment) or to the *Bam*HI site at about -600 base pairs (bp) (HB fragment) were shown to contain full enhancing activity. The *Bst*XI to *Eco*RI fragment (XR), containing the first E_{β} gene exon and a portion of the first intron, had no detectable activity. We have previously reported that two short sequences present upstream of the E_{β} gene promoter are conserved in the corresponding regions of the murine E_{α} and DR_{α} genes⁵ (see Fig. 3). As the HB and HX fragments worked equally well in the enhancer assay, even though these sequences were not contained in the HB fragment, we conclude that these elements are not necessary for enhancing activity.

Table 1 Relative transformation frequencies of pSV2gpt and derivative plasmids

Plasmid	Transfected cells		
	A20-2J	J558L	L
a			
pSV2gpt	1.0		
pSER	<0.1		
pSER-13 + pSER-13 R	0.55		
pSER-0.7 + pSER-0.7 R	<0.1		
pSER-1.9 + pSER-1.9 R	<0.1		
pSER-12 + pSER-12 R	<0.1		
pSER-8.6 + pSER-8.6 R	0.05		
pSER-4.1 + pSER-4.1 R	1.1		
b			
pSV2gpt	1.0	1.0	1.0
pSER	<0.05	0.02	0.05
pSER-X _{2/3} (heavy chain)	1.9	1.5	0.05
pSER-4.1 R	1.6	<0.02	0.04
pSER-4.1	1.6		
pSER-HX R	1.9		
pSER-HX	1.9		
pSER-BX R	0.17		
pSER-BP R	<0.05		
pSER-HB R	2.0	<0.02	0.05
pSER-HB	2.0		
pSER-PB R	<0.05		
pSER-HP R	<0.05		
pSER-AA R	<0.05		
pSER-XR R	<0.05		

Cells were transfected by protoplast fusion as described⁸ and plated at 2×10^3 cells per well (A20-2J), 10^4 cells per well (J558L) or 10^4 and 10^5 cells per 100 mm dish (L cells). Selective medium contained mycophenolic acid at $1 \mu\text{g ml}^{-1}$ (A20-2J), $6 \mu\text{g ml}^{-1}$ (J558L) or $25 \mu\text{g ml}^{-1}$ (L cells). The transformation frequencies were normalized to a value of 1.0 which was assigned to the frequency obtained with plasmid pSV2gpt (10^{-4} for A20-2J, 4×10^{-4} for J558L and 2×10^{-3} for L cells). **a**, Four *Eco*RI fragments (13, 0.7, 1.9 and 12 kb long) covering ~28 kb of the E_{β} gene and its flanking regions (see Fig. 1) were cloned in both orientations into the *Eco*RI site of plasmid pSER. Bacterial cultures containing plasmids with the same fragment in both orientations were mixed before protoplast preparation. The positive 13-kb *Eco*RI fragment was subdivided into an 8.6-kb *Hind*III fragment and a 4.1-kb *Hind*III-*Eco*RI fragment. These fragments were cloned by blunt-end ligation into the *Eco*RI site of plasmid pSER and tested for transformation efficiency against A20-2J. **b**, The 4.1-kb *Hind*III-*Eco*RI fragment was further subdivided as described in the text. Each plasmid was tested at least three times using constructs containing the test DNA fragment in both orientations. For most fragments, only the reverse orientation with respect to gpt transcription is shown. Plasmid nomenclature: see Fig. 1 for the restriction fragments inserted into the *Eco*RI site of plasmid pSER. The letter R refers to the reverse orientation used for the transformation data shown.

Subfragments of the 2.0-kb HB fragment were negative in the pSER assay (Table 1). These include the fragments produced by cleavage with *Pvu*II (HP and PB) and a fragment spanning the *Pvu*II site (AA). Thus it seems that the sequences required for enhancer activity are scattered over the 2.0-kb HB fragment.

Next we tested the recombinant plasmids that gave positive results in A20-2J cells for enhancing activity in other murine cell types (Table 1). No activity was detected when the 4.1-kb or HB fragment recombinants were used to transfect fibroblasts (L cells), indicating that this enhancing activity is tissue-specific. These same plasmid constructs also gave negative results in a myeloma J558L, a tumour of plasma cells which are terminally differentiated B-lineage cells not expressing class II antigens. In contrast, the pSER recombinant containing the immunoglobulin heavy-chain enhancer⁸ was positive in both the B-lymphoma (A20-2J) and myeloma lines. We conclude that the cell type-specific expression of the E_{β} gene is due, at least in part, to the functionality of its associated enhancer element.

In order to prove more directly that the E_{β} gene carries an associated enhancer element, we inserted the HB fragment into

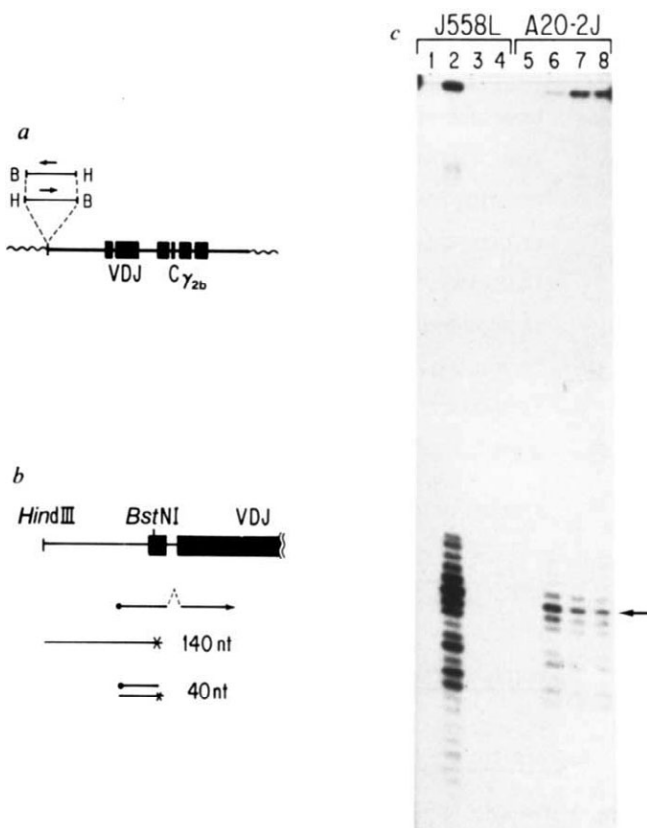


Fig. 2 Enhancement of $\gamma 2b$ heavy-chain mRNA transcription in J558L myeloma and A20-2J lymphoma cells. **a**, Diagram of plasmid pSV- $\gamma 2bX_{2/4}$ (ref. 8) and the site of insertion of the 2.0-kb HB fragment from the 5' flanking region of the E_{β} gene. This plasmid contains a functionally rearranged $\gamma 2b$ heavy-chain immunoglobulin gene but the heavy-chain enhancer has been removed (see Gillies *et al.*,⁸ for details). The HB fragment was inserted in both orientations ~1.4 kb upstream of the $\gamma 2b$ promoter. **b**, Diagram of the S₁ nuclease protection assay used to measure $\gamma 2b$ mRNA levels. A single-stranded *Hind*III to *Bst*NI fragment (140 nucleotides, nt) was hybridized to total cell RNA (20 μg) from transfected and control cells. The 40-nucleotide major protected fragment is indicated. **c**, Electrophoretic analysis of fragments protected by RNA from cells transfected with plasmid pSV- $\gamma 2bX_{2/4}$ (lanes 1 and 5) and recombinants containing the heavy-chain enhancer (lanes 2 and 6) or the E_{β} HB fragment in the normal (lanes 3 and 7) or reversed orientations (lanes 4 and 8). An arrow indicates the 40-nucleotide major protected fragment.

Sequence of the 5' flanking region and exon 1 of the mouse E_{β}^d gene. The sequencing strategy is shown in Fig. 1 and was performed under procedures¹⁹. Exon 1 is underlined with a thick line. Boxed sequences include two conserved sequence blocks shared with E_{α} and human DR_{α} genes¹⁹ (nucleotides 2,551 and 2,583), the 'CCAAT' box at 2,610 and the 'TATA' box at 2,646. Short sequences resembling 'core' elements are underlined and their orientations with respect to E_{β} transcription are indicated with arrows. Three long stretches of C-G-pyrimidine asymmetry are also underlined and begin at nucleotides 13, 1,001 and 1,411. Stretches of alternating purines and pyrimidines are indicated with a dotted line.

in either orientation (Fig. 2). The results show that J558L cells transfected with the construct containing the heavy-chain enhancer contained high levels of $\gamma 2b$ mRNA (lane 2 of Fig. 2c), confirming the results of our previous report⁸. In contrast, no $\gamma 2b$ mRNA could be detected in J558L cells transfected with the constructs containing the E_{β} HB fragment in either orientation (lanes 3 and 4), even with much longer exposures of the autoradiogram (not shown). A lower level of $\gamma 2b$ mRNA (relative to J558L cells) was detected in A20-2J cells transfected with the plasmid construct containing the heavy-chain enhancer fragment (lane 6). In contrast to the results with J558L cells, A20-2J cells transfected with the E_{α} HB fragment constructs contained

levels of $\gamma 2b$ mRNA that were comparable to that seen in the same host cell (A20-2J) with the heavy-chain enhancer (lanes 7 and 8).

Transfected cell lines were analysed further by Southern blotting to determine the plasmid copy numbers (data not shown). Except for the J558L line, obtained by transfection with the heavy-chain enhancer-containing plasmid (one or two copies per cell), all of the lines of both A20-2J and J558L contained a high copy number (about 40 copies per cell). This dramatic effect of the heavy-chain enhancer on the plasmid copy number in J558L transfectants, obtained by gpt selection, was described previously⁸. When the level of $\gamma 2b$ mRNA (determined by S_1 nuclease protection; Fig. 2) is normalized to the number of gene copies, it seems that the strength of the heavy-chain enhancer in J558L as opposed to A20-2J is even greater than the differences seen in the intensity of the S_1 -protected bands (Fig. 2, lanes 2 and 6). Note, however, that factors such as mRNA stability or differential promoter strength may also affect the steady-state levels of $\gamma 2b$ mRNA in the two cell types. Nonetheless, these results are in agreement with the data obtained from the pSER transformation assay (Table 1) and demonstrate that the heavy-chain sequences function as an enhancer in both B cells and plasma cells, albeit with different strength, while the E_β HB fragment functions as an enhancer in the B lymphoma expressing class II antigens but not in the myeloma not expressing these antigens. Thus, functioning of this enhancer correlates with the expression of the E_β gene in the terminal stages of B lymphoid cell differentiation.

DNA sequence analysis of the 5' flanking regions of the E_β gene revealed no significant similarity to the heavy-chain enhancer (Fig. 3). The enhancer 'core' sequence, 5'-GTGG^{AAA}G-3' (ref. 14), common to most known viral and cellular enhancers, occurs once in this region around nucleotide 2,150 and is oriented in the direction opposite that of E_β transcription. Three 'core-like' sequences, each containing a C residue between the third and fourth G residues of the core consensus sequence, are located between nucleotides 551 and 767. These sequences are spaced approximately 100 bp apart and each successive repeat contains an additional A or T between the third and fourth G residue than the one before it. A fourth 'core-like' sequence containing five A or T residues is located around nucleotide 1,100. All four 'core-like' sequences are contained within the *Hind*III-*Pvu*II (HP) and *Ava*II-*Ava*II (AA) fragments which by themselves have no enhancer activity (Table 1).

The most striking feature of the sequence in this region is the dinucleotide repeats beginning with 24 TC repeats near the *Hind*III site. This is followed by a track of alternating purine-pyrimidines which extends for approximately 170 bp with only five single base pair interruptions. Shorter stretches are also found around nucleotides 250 and 800. Such sequences are known to have the potential of forming left-handed Z-DNA¹⁵, however this property is not by itself sufficient for the enhancing activity of the E_β enhancer as these sequences are located within the inactive HP fragment.

There are two additional tracks of purine-pyrimidine asymmetry—a GA repeat between nucleotides 1,001 and 1,054 and a TC repeat between nucleotides 1,411 and 1,450. It is not yet known whether such sequences have a role in E_β enhancer function. It should be noted, however, that the (TC)₂₄-(GA)₂₇-(TC)₂₀ tracts are all contained in the HB fragment (the smallest fragment with full enhancing activity) whereas the inactive HP, PB and AA fragments contain only one or two. Clearly, the individual contributions of the elements described above as well as those involved in the strict cell-type specificity of this enhancer will require much more refined analyses.

The results presented above provide another example of a cell type-specific cellular enhancer element which is likely to play an important part in the regulated expression of the associated gene. It will be interesting to determine to what extent the expression of other eukaryotic genes is controlled by enhancers or similar *cis*-acting elements such as the upstream activation sequences (UASs)¹⁶ of yeast and the 'modulator' sequences of

the sea urchin histone¹⁷, human insulin and chymotrypsin genes¹⁸.

We thank Ms Lena Angman for technical help and Ms Eleanor Basel for secretarial assistance. V.F. is supported by a fellowship from the Cancer Research Institute. This work was supported by grants AI-17879 (S.T.) and CA-14051 (a core grant to S. Luria) from the NIH.

Received 1 May; accepted 14 June 1984.

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Graded and nonlinear mechanical properties of sensory hairs in the mammalian hearing organ

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It is generally agreed that frequency selectivity of the mammalian hearing organ is mainly due to a graded elasticity of the basilar membrane¹. Recent measurements of basilar membrane motion^{2,3}, hair cell receptor potentials⁴ and neural tuning curves⁵ show that frequency selectivity can be extremely sharp. It has been suggested that in non-mammalian species there are additional tuning mechanisms in the sensory hair cells themselves, either by virtue of their electrical membrane properties⁶ or through a gradation in length of their sensory hairs^{7,8}. Indeed, sensory hair mechanical tuning has been demonstrated in the lizard⁹. We have investigated the mechanical properties of sensory hair bundles in the guinea pig organ of Corti, and report here that hair-bundle stiffness increases longitudinally towards the high-frequency end of the cochlea, decreases radially towards the outer rows of cells, and is greater for excitatory than for inhibitory deflection. On the basis of these findings, we suggest that sensory hairs confer frequency-specific, nonlinear mechanical properties on the hearing organ.

The preparation used in our experiments consisted of a segment (about three-quarters of a turn) of the cochlear partition from the second (T2), third (T3) or fourth (T4) turn of the cochlea, carefully dissected free and maintained at room temperature in tissue culture medium (L-15 Leibovitz liquid medium; Gibco Bio-Cult). After the removal of the tectorial membrane in incident light, the coil was transferred to a microscope chamber where it was held horizontally and rotated to select the desired cells and to allow appropriate alignment of hair bundles on the inner (IHC) and outer (OHC) hair cells with respect to a measuring probe. The probe comprised a

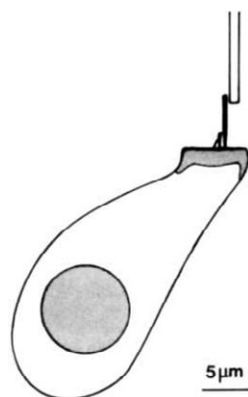


Fig. 1 Schematic illustration of the way in which a quartz glass fibre is applied to the stereocilia of an inner hair cell. Pushing towards the left, sufficient force is applied to move the tip of the tallest row of stereocilia by $1\ \mu\text{m}$. The degree of bending of the glass fibre gives a measure of sensory hair stiffness. The measurements were performed on isolated coils of guinea pig organ of Corti.

vertically-held quartz glass fibre $\sim 1\ \mu\text{m}$ in diameter and 500–700 μm long. Force was applied by pushing the fibre perpendicularly against the row of stereocilia in a selected hair cell (Fig. 1) until the row was displaced by $1\ \mu\text{m}$ (Fig. 2). The stiffness of the hair bundle (the force required to produce a $1\ \mu\text{m}$ displacement of the bundle) was determined from the bending of the probe which had been previously calibrated for stiffness. Signs of trauma to the sensory hairs and of degeneration in the cell bodies could be detected under the microscope and hence such regions were avoided. A full description of the criteria used to assess viability is given elsewhere¹⁰.

Measurements of inner hair cell membrane potential (usually $>60\%$ of the *in vivo* values) were used as further controls to determine the physiological condition of the preparation. Stiffness values and membrane potentials remained constant throughout the lifetimes of the preparations (3 to 4 h).

We observed that the stereocilia within a row held together as if linked to one another; when force was applied at one point, neighbouring segments of the row were pulled along in the displacement. The stereocilia do not bend but pivot at the base; they are brittle and fracture at the base when displaced by more than $2\ \mu\text{m}$. The stiffness of sensory hairs in all four rows of the three coils was measured in 130 cells from 15 preparations. The data and a statistical analysis are given in full elsewhere¹¹. The results are summarized graphically in Fig. 3, where each measurement is the average of over 23 readings from at least four cells at each location. There is an increase in stiffness of the sensory hair bundle towards the base of the cochlea, with OHC exhibiting a greater increase than IHC: for example, the stiffness values for the first row of OHC are 0.78 ± 0.22 , 1.22 ± 0.40 and $3.47 \pm 0.70\ \text{dyn cm}^{-1}$ in turns T4, T3 and T2, respectively. There is also a gradation in stiffness radially among OHC, stiffness decreasing from the first to the third row.

The sensory hairs also vary in length in the different rows. If the sensory hairs act as levers with elastic hinges at their bases, the measured stiffness of the sensory hairs should be proportional to the product of the angular deflection and the inverse of the length. The lengths of the sensory hairs were measured in the three cochlear turns, and the ratio of the angular deflection to the length was calculated and plotted in Fig. 3; clearly, the variation in the length of sensory hairs could account for some, but not all, of the variation in torque stiffness (it is assumed that the pivot has constant angular stiffness).

Another significant finding is that the stiffness is greater for force applied in the direction that would, in physiological terms, be excitatory (that is, away from the centre of the coil) than in the inhibitory direction. The excitatory/inhibitory stiffness ratios of 1.9 ± 0.7 ($n=20$) for IHC and 2.5 ± 1.3 ($n=20$) for OHC from T3 are significantly different ($P < 0.1$). As these estimates

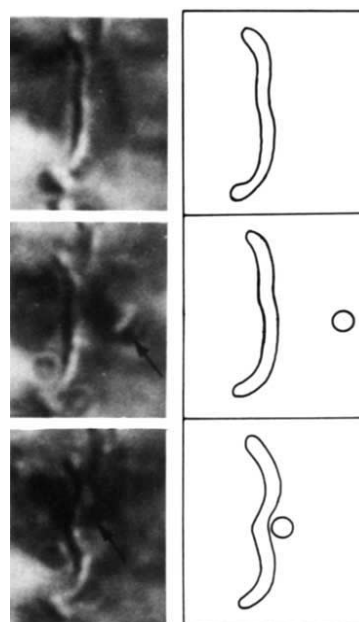


Fig. 2 Photograph (from above) of a row of stereocilia belonging to an inner hair cell being approached and pushed by the glass fibre probe (arrow) during an experiment. The sequence is shown schematically on the right.

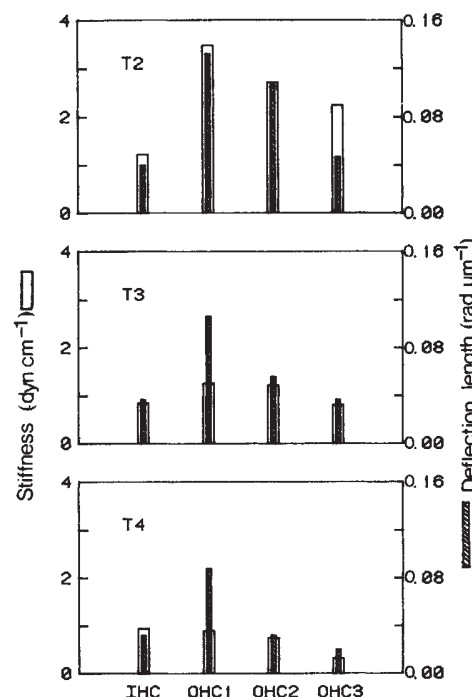


Fig. 3 Open bars, stiffness of sensory hairs of inner hair cell (IHC) and outer hair cells of rows 1 (OHC1), 2 (OHC2) and 3 (OHC3) in the second (T2), third (T3) and fourth (T4) turns of the guinea pig cochlea. Hatched bars, ratio of sensory hair angular deflection to length at the same locations. The measurements were made in the excitatory direction.

of excitatory/inhibitory ratios were obtained at greater than normal physiological deflections, it is not possible to estimate whether the observed nonlinearities are present at low stimulus levels.

The results presented here were obtained from *in vitro* preparations at room temperature ($21\text{--}23\ ^\circ\text{C}$), and by the application of relatively large-amplitude¹² static displacements. Although these conditions are highly non-physiological, the experiments

may reveal qualitative properties of sensory-hair micro-mechanics that also apply in the dynamic physiological situation. The implications of our results are that sensory cells in different coils may be tuned, just as is the basilar membrane, and contribute to frequency selectivity of the organ through coupling of the sensory hairs of outer hair cells to the tectorial membrane. The observation of asymmetrical stiffness in the sensory hair in response to alternating stimulus directions may relate to another question at the heart of contemporary cochlear physiology—that is, the nature of the mechanical nonlinearity seen at low and moderate sound pressure levels³. The structure responsible for this phenomenon is known to be involved in that part of cochlear transduction which accounts for the finest tuning and which is biologically vulnerable. Furthermore, it has been implicated in generating the nonlinearities seen in active components of the acoustic mechanical response^{13,14}.

The above results suggest that the function of the mammalian hearing organ as a highly-tuned mechanoreceptor depends not only on frequency selectivity in the basilar membrane but also on tuning and nonlinear properties in the sensory hairs of the receptor cells, and on their coupling to the tectorial membrane.

This work was supported by grants to Å.F. from the Swedish MRC (04X-0246), the Söderbergs Foundation and Stiftelsen Tysta Skolan, and to D.S. from the USPHS (NS 00255, NS 08193).

Received 3 February; accepted 8 June 1984.

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Do general anaesthetics act by competitive binding to specific receptors?

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Most proteins are insensitive to the presence of anaesthetics at concentrations which induce general anaesthesia, while some are inhibited by certain agents but not others¹. Here we show that, over a 100,000-fold range of potencies, the activity of a pure soluble protein (firefly luciferase) can be inhibited by 50% at anaesthetic concentrations which are essentially identical to those which anaesthetize animals. This identity holds for inhalational agents (such as halothane, methoxyflurane and chloroform), aliphatic and aromatic alcohols, ketones, ethers and alkanes. This finding is all the more striking in view of the fact that the inhibition is shown to be competitive in nature, with anaesthetic molecules competing with substrate (luciferin) molecules for binding to the protein. We show that the anaesthetic-binding site can accommodate only one large, but more than one small, anaesthetic molecule. The obvious mechanism suggested by our results is that general anaesthetics, despite their chemical and structural diversity, act by competing with endogenous ligands for binding to specific receptors.

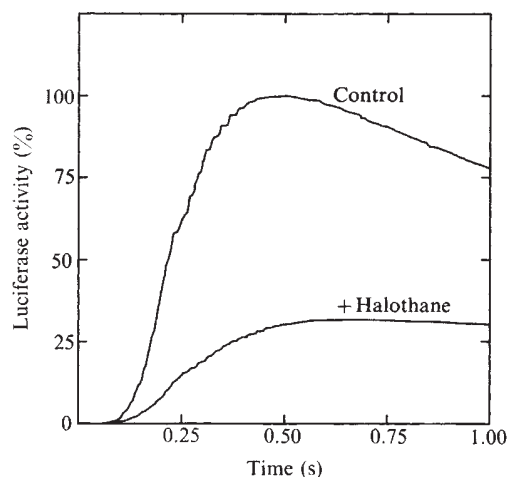


Fig. 1 Inhibition of luciferase activity at surgical levels of halothane. The curves show luciferase activity (light intensity) as a function of time, after the rapid mixing of luciferase and luciferin with ATP. For the lower curve, the final concentration of halothane was 0.45 mM, equivalent to a partial pressure of 0.0073 atm. For all experiments reported here, 2.5 ml of a buffered ATP solution in an air-driven syringe was rapidly injected into a vial containing 5 ml of a buffered solution of luciferase, luciferin and $MgSO_4$, with or without anaesthetic. Final concentrations of ATP and $MgSO_4$ were 2 mM and 6.7 mM, respectively, in 25 mM glycylglycine buffer at pH 7.8 and a temperature of $22 \pm 1^\circ C$. The final concentration of luciferase was of the order of 10 nM. Variable concentrations of luciferin and anaesthetics were used. For this figure, the final luciferin concentration was 2 μM . The effects of anaesthetics on luciferase were shown to be reversible by dilution of the anaesthetic, after which, activities returned to within (at worst) 10% of control values. The time to maximum response increased slightly in the presence of all anaesthetics. This was probably due to the competitive nature of the inhibition—the same effect was observed when the luciferin concentration was lowered. Light intensity was measured with a photomultiplier tube and the output stored on a digital transient recorder. Desiccated firefly lanterns and D-luciferin were supplied by Sigma. Using these lanterns and the procedure of Branchini *et al.*⁸, we obtained the pure crystalline luciferase which we used in all our experiments.

In order to test the hypothesis that general anaesthetics act directly on protein, it is essential to study a pure protein, completely free of lipid, so that any effects observed can be interpreted unambiguously in terms of anaesthetic–protein interactions. Previous work with whole cells^{2–4} and partially purified extracts^{5–7} suggested that the light-emitting luciferase proteins would be good candidates. We purified the luciferase enzyme from the North American firefly *Photinus pyralis* using affinity chromatography⁸ and obtained a pure crystalline protein, completely free of the substrate luciferin (see Fig. 1 legend for details). Firefly luciferase combines with its substrate luciferin (molecular weight 280), in the presence of ATP, Mg^{2+} and O_2 , to give a photon of light⁹. When the reactants are rapidly mixed, an initial burst of light is followed by a slow decay (see Fig. 1).

The maximum rate of light production was extremely sensitive to the presence of anaesthetics (see Fig. 1). We first measured ED_{50} concentrations for a wide range of general anaesthetics at a luciferin concentration equal to the K_m of the uninhibited enzyme, in conditions where ATP, Mg^{2+} and O_2 were not rate-limiting. The resulting anaesthetic potencies are plotted in Fig. 2 against the corresponding general anaesthetic potencies for whole animals. These data show a striking correlation between luciferase and whole animal potencies over a 100,000-fold range, with all points lying to within a factor of about 2 from the line of identity. This identity is all the more remarkable in view of the diversity of anaesthetics used.

We next determined the mechanism of anaesthetic inhibition by measuring enzyme activities over a range of both luciferin and anaesthetic concentrations. We found the inhibition to be

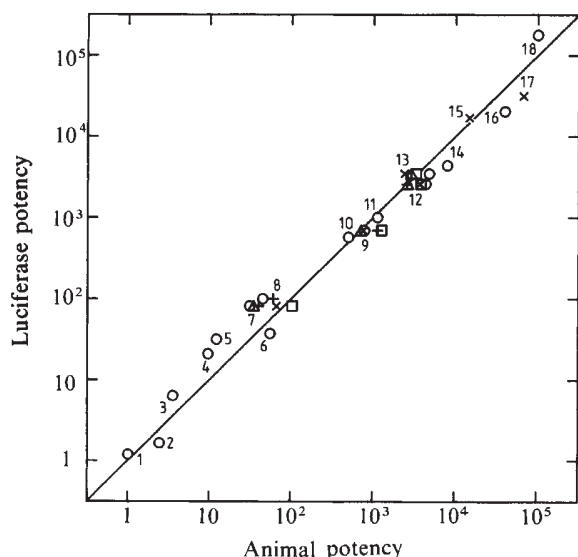


Fig. 2 Identity of general anaesthetic concentrations needed to anaesthetize whole animals and to inhibit luciferase activity by 50%, for a diverse range of simple anaesthetics over a 100,000-fold range of potencies. The line is the line of identity. The data are plotted as potencies, defined as reciprocals of aqueous ED_{50} concentrations (M). Symbols: $\square$, man; $\times$, mouse; $+$, newt; $\circ$, tadpole; $\triangle$, goldfish. For the luciferase measurements, the luciferin concentration was equal to the K_m of the uninhibited enzyme. In our conditions, the average uninhibited K_m was $14 \mu\text{M}$ luciferin. Whole animal ED_{50} concentrations were obtained from the following sources: man (compounds 7, 10, 12, 13: Table 6 of ref. 18); mouse (compounds 7, 10, 12, 13: Table 3 of ref. 19, and compounds 15, 17: Table 11 of ref. 20); newt (compounds 7, 8, 10, 12: ref. 21); tadpole (compounds 1–8, 10, 11, 14: Table 5 of ref. 20, compounds 16, 18: Table 4 of ref. 20, and compounds 9, 12, 13: ref. 22); and goldfish (compounds 7, 10, 12, 13: ref. 23). When the ED_{50} was given as a partial pressure, it was converted into an aqueous concentration as described previously¹⁴. For compounds 15 and 17, the gaseous concentrations given in Table 11 of ref. 20 were converted to aqueous concentrations using Henry's law constants at 37°C , calculated using the data in Table 4 of ref. 24. Anaesthetics are referred to as follows: 1, methanol; 2, ethanol; 3, acetone; 4, *n*-propanol; 5, butanone; 6, paraldehyde; 7, diethyl ether; 8, *n*-butanol; 9, benzyl alcohol; 10, chloroform; 11, *n*-hexanol; 12, halothane; 13, methoxyflurane; 14, *n*-octanol; 15, pentane; 16, *n*-nonanol; 17, hexane; 18, *n*-decanol.

purely competitive in nature, with the anaesthetic molecules competing with luciferin molecules for binding to the protein. This is shown for halothane in Fig. 3, where it can be seen that the anaesthetic increases the apparent Michaelis constant (K_m^{app}) but has no significant effect on the maximum activity (V_{max}).

Further information on the anaesthetic-protein interaction is contained in the values of the slopes of Fig. 3. Each slope provides an accurate value for $K_m^{\text{app}}/V_{\text{max}}$, and these values (each divided by the slope in the absence of anaesthetic) are plotted against anaesthetic concentration in Fig. 4a. The function $f(A)$, so defined, is the factor by which the uninhibited K_m increases in the presence of an anaesthetic at a concentration A . Interestingly, the data points do not lie on a straight line ($P < 0.001$), but fit a quadratic equation very accurately. This immediately suggests that two molecules of halothane are involved in the inhibition. The simplest way to explain our data is to assume that two halothane molecules can bind independently to the protein, each with a dissociation constant K_i , but that the presence of only one is sufficient to cause inhibition. It is then easy to show that $f(A) = (1 + A/K_i)^2$, so that a plot of the square root of $f(A)$ against A would give a straight line with a slope of $1/K_i$. Figure 4a shows that the halothane data do indeed give an excellent straight line when plotted in this way, giving a value of $K_i = 0.54 \text{ mM}$.

We next investigated whether the two halothane molecules were acting at two well-separated sites, or whether both sites were contained within a single region of limited size, such as a small hydrophobic pocket. If the latter were true, one would expect that, with progressively larger anaesthetic molecules, a point would be reached at which only a single molecule could be accommodated. We therefore studied the effects of the homologous series of *n*-alcohols, from methanol to *n*-decanol. We found that for the lower members of the series, up to and including hexanol (see Fig. 4b), the inhibition resembled that of halothane in that more than one anaesthetic molecule was involved. However, for octanol and decanol, the pattern of inhibition suddenly changed. For these large anaesthetics it was clear that only a single molecule was involved in the inhibition (see, for example, Fig. 4c). The plot of $f(A)$ against A is a straight line (even up to very high anaesthetic concentrations where $f(A) = 14$; Fig. 4c), whereas the square root plot is no longer linear. This is consistent with a scheme in which only a single anaesthetic molecule binds, for which $f(A) = 1 + A/K_i$. It thus appears that the anaesthetic molecules bind to the protein at a site of limited size.

Some idea as to the size of the site can be obtained from our data. Because the site can accommodate two molecules of hexanol but only one of octanol, the minimum volume of the site must be roughly that of two hexanol molecules, or about 250 ml mol^{-1} . (Interestingly, a hydrophobic pocket of a similar size has also been found in β -lactoglobulin^{10,11}.) The space-filling models in Fig. 4d demonstrate the similarity between the molecular sizes of two hexanol molecules, two halothane molecules, one decanol molecule and the substrate luciferin. Taking these size considerations together with the competitive nature of the inhibition, our results strongly suggest that the anaesthetic molecules are binding within the luciferin site. This

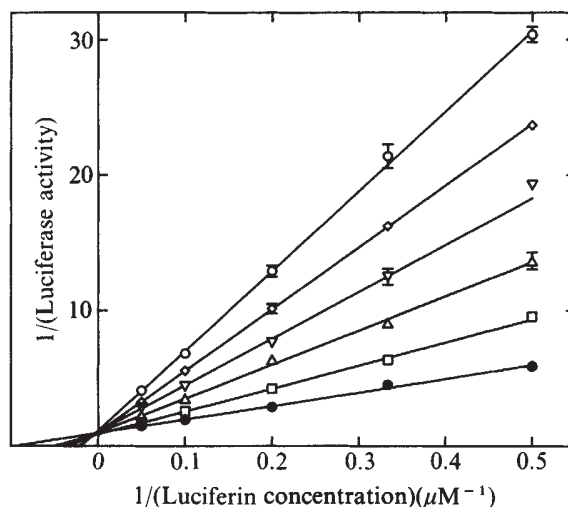


Fig. 3 Halothane inhibits luciferase activity by competing with its substrate luciferin. The reciprocal of the maximum rate of light production (the peak height) is plotted, in relative units, against the reciprocal of the luciferin concentration, at various concentrations of halothane: $\bullet$, control; $\square$, 0.15 mM; $\triangle$, 0.30 mM; ∇ , 0.45 mM; $\diamond$, 0.60 mM; $\circ$, 0.75 mM. Data points are the means of multiple measurements, and the error bars give the maximum spread of the data. Where error bars are not shown, the spread of the data was less than the size of the symbol. The straight lines were fitted to the data using the method of least squares, with weighting factors proportional to the square of the enzyme activities²⁵. Essentially identical lines were obtained using distribution-free procedures²⁵. Halothane was supplied as Fluothane (ICI); the stabilizer, thymol, which is present in Fluothane, did not inhibit luciferase activity at the concentrations achieved in these experiments.

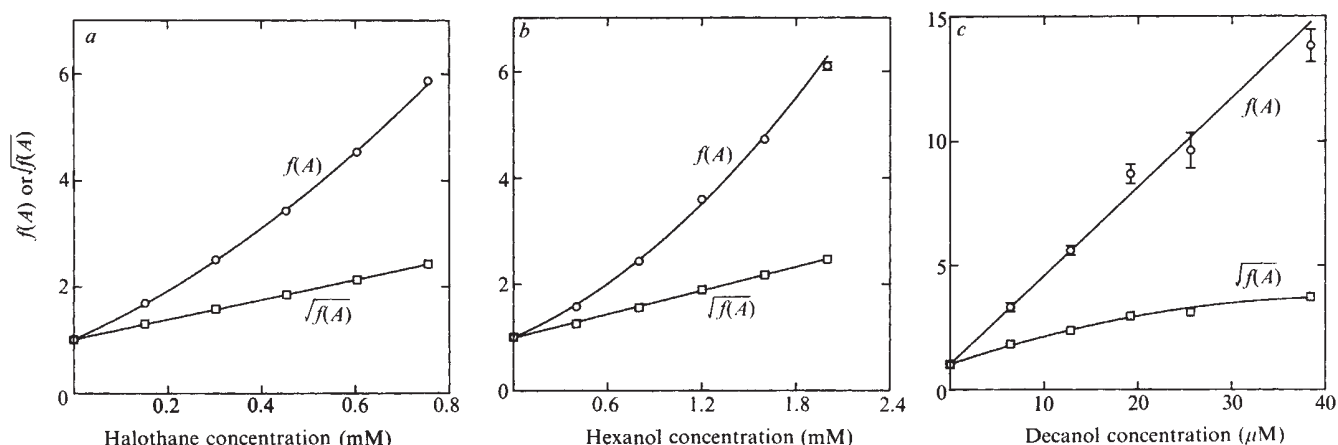


Fig. 4 The anaesthetic binding site on luciferase can accommodate two molecules of halothane, two molecules of hexanol, but only one molecule of decanol. Details of the competitive inhibition are revealed by analysing the slopes of the double-reciprocal plots. *a*, Halothane; *b*, *n*-hexanol; *c*, *n*-decanol. The function $f(A)$, where A is the anaesthetic concentration, is the ratio of the slopes of double-reciprocal plots (see, for example, Fig. 3) in the presence and absence of anaesthetic. Since $V_{\max}$ is not affected by anaesthetics, $f(A)$ is thus the factor by which the uninhibited K_m increases in the presence of anaesthetic. Alternatively, $f(A)$ can be taken as the ratio of luciferin concentrations which would be required to produce the same activity in both the presence and absence of anaesthetic. Error bars give the standard errors in $f(A)$ and $\sqrt{f(A)}$; where error bars are not shown, the standard error was less than the size of the symbol. The lines were calculated using the method of least squares, with weighting factors proportional to the reciprocal of the squares of the standard errors. From the slopes of the straight lines, the inhibition constants K_i were calculated to be 0.54 mM for halothane, 1.3 mM for *n*-hexanol and 2.8 μ M for *n*-decanol. *d*, Space-filling models showing, from left to right, the relative sizes of two molecules of halothane, two molecules of *n*-hexanol, one molecule of *n*-decanol and one molecule of the substrate luciferin.

conclusion is supported by the finding that luciferin binds in a hydrophobic pocket^{12,13}, which presumably extends to the water-protein surface. Such a binding site might naturally be expected to have both polar and apolar parts, an apparent characteristic of the general anaesthetic target site¹⁴.

The possibility that a wide range of structurally and chemically dissimilar molecules might cause general anaesthesia by competing at specific sites on proteins for the binding of an endogenous ligand might, at first sight, seem very implausible. How could a site which specifically binds a particular ligand accommodate the diverse range of molecules that cause general anaesthesia? While a detailed answer to this question must await future work, our luciferase data nonetheless show clearly that such sites on proteins do exist.

The molecular mechanism of general anaesthesia may well involve a number of different protein-anaesthetic interactions¹⁵⁻¹⁷. Our results, however, emphasize the central role competitive inhibition may have. The diverse functions, and even the identities, of many neuropeptides and transmitters in the central nervous system are only beginning to be elucidated, but it is easy to imagine that general anaesthetics may act by competing with such endogenous ligands. (For example, a small peptide with one or more hydrophobic amino acid residues would be an obvious candidate.) This raises the intriguing possibility that if the endogenous ligands can be identified, it may be possible to design general anaesthetic agents that are far more specific than those currently available.

We thank Professor Alan Fersht for help throughout this work, John Akins for technical assistance, Mike Harris and

Susan Watts for constructing some of the apparatus, Nick Jackson for the photographic work, Ian Clark for the artwork and Professor David Blow for helpful comments on the manuscript. This research was supported by grants from the MRC and the BOC Group, Inc.

Received 28 February; accepted 1 June 1984.

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Chiral selection in poly(C)-directed synthesis of oligo(G)

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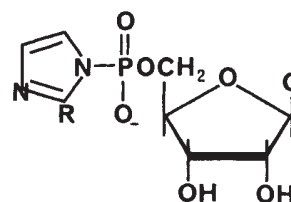


Fig. 1 Structure of guanosine 5'-phospho-2-methylimidazole ($R = \text{CH}_3$, as used in system I) and guanosine 5'-phosphorimidazole ($R = \text{H}$, as used in system II).

Theories of the origin of optical asymmetry in living systems place fundamental importance on the amplification of optical asymmetry by an autocatalytic process¹⁻³. The replication of a polynucleotide is one obvious choice for such an autocatalytic growth mechanism. If an optically homogeneous polynucleotide could replicate by directing the polymerization of monomers of the same handedness, while excluding monomers of the opposite handedness, its chiral descendants would come to dominate what was once an achiral environment. Recently, two highly efficient template-directed reaction systems have been developed for the oligomerization of activated guanosine mononucleotides (Fig. 1) on a poly(C) template^{4,5}. The synthesis of L-guanosine 5'-mononucleotide⁶ makes it possible to study chiral selection in these systems. We report here that poly(C)-directed oligomerization of activated guanosine mononucleotides proceeds readily if the monomers are of the same optical handedness as the template, and is indeed far less efficient if the monomers are of the opposite handedness. However, in template-directed reactions with a racemic mixture, monomers of the opposite handedness to the template are incorporated as chain terminators at the 2'(3') end of the products. This inhibition raises an important problem for many theories of the origin of life.

System I involves the poly(C_D)-directed oligomerization of guanosine 5'-phospho-2-methylimidazole (2-MeImpG) in the presence of Mg^{2+} . D- and L-2-MeImpG were synthesized from their respective 5'-nucleotides⁷, and all other procedures were carried out as described previously⁵. Figure 2 shows HPLC elution profiles of the product distribution obtained from the various reactions.

In the absence of a template, the D and L isomers of 2-MeImpG, of course, give identical mixtures of products (Fig. 2a,b); dimers and smaller amounts of trimers are formed. The presence of a poly(C_D) template leads to highly efficient oligomerization of D-2-MeImpG (Fig. 2c). The major peaks in the HPLC profile correspond to all 3'-5'-linked oligo(G)s ranging in length from the dimer to about the 20-mer. Poly(C_D)-directed oligomerization of L-2-MeImpG is far less efficient (Fig. 2d), though a comparison of Fig. 2b and d shows that there is a well-defined template effect for the L isomer. The major template-directed products are GppG, (pG)₂ and Gp(pG)₂, and there is also a substantial increase in the yields of Gp(pG)₃ and Gp(pG)₄.

The HPLC profiles in Fig. 2e and f show the product distribution obtained in template-directed reactions with a racemic

mixture of 2-MeImpG. Clearly, the L isomer substantially reduces the efficiency of oligomerization of the D isomer. Furthermore, the single major series of peaks corresponding to all 3'-5'-linked oligo(G)s has been replaced by complex groups of up to four peaks, suggesting that oligomers containing both D- and L-nucleotides have been produced. The template-directed reaction with L-2-MeImpG alone suggests that once an L-monomer has been incorporated into a newly synthesized oligomer, the efficiency of subsequent monomer incorporation is greatly reduced. Most of the G_L residues present in optically mixed chains, therefore, are likely to be located at either the 5' or the 2'(3') terminus.

To quantify the inhibition of oligomerization by L-2-MeImpG and to determine the nature of the oligomeric products, we carried out reactions using ¹⁴C-labelled D-2-MeImpG. The data (see Table 1) show that the yield of oligomers containing radioactive G_D residues is reduced by more than a factor of two when an equal amount of L-2-MeImpG is added to the reaction mixture.

The oligomeric products of tetramer length and longer were collected by paper chromatography and degraded by exhaustive alkaline hydrolysis. The hydrolysed materials were applied to a paper chromatogram together with authentic markers, and the relative yields of ¹⁴C-containing degradation products were obtained⁷. In this way, we confirmed that 95% of the products obtained with D-2-MeImpG are standard oligonucleotides and the remaining 5% are oligonucleotides with an additional pG residue attached by a pyrophosphate bond at the 5' terminus. The analysis of reactions using 0.05 M DL-2-MeImpG indicated that 80% of the chains were capped at their 5' terminus with a pyrophosphate involving a G_L residue and at least 55% of the chains had a G_L residue attached to their 2'(3') terminus by means of a 2'-5' or 3'-5' internucleotide bond. The corresponding yields for the reaction with 0.1 M DL-2-MeImpG were ~50% for 5'-pyrophosphate termination and 50% for 2'(3') termination with a G_L residue.

The results of alkaline hydrolysis suggest that there should be at least four series of peaks in the HPLC elution profile of template-directed reactions with racemic 2-MeImpG. The reaction with 0.05 M DL-2-MeImpG is expected to produce two major series of peaks, corresponding to G_Lp(pG_D)_n and G_Lp(pG_D)_npG_L, and two minor series of peaks corresponding to (pG_D)_n and (pG_D)_npG_L; for oligomers of trimer length and longer this seems to be the case (Fig. 2c). The reaction with 0.1 M DL-2-MeImpG is expected to produce all four series of peaks in comparable amounts; this seems to be the case also, although the peaks are incompletely resolved at some oligomer lengths (Fig. 2f). Co-injection with authentic (pG_D)_n confirms that the all-G_D oligomers appear as a minor series of peaks in Fig. 2e and as a major series of peaks in Fig. 2f. The fact that no more than four prominent series of peaks are present in either profile indicates that optically mixed oligomers containing G_L residues in nonterminal positions are produced in only small amounts.

Poly(C_D)-directed oligomerization of D-2-MeImpG is not inhibited by the addition of activated adenosine, cytidine or uridine mononucleotides to the reaction mixture⁵. Therefore, the inhibition by L-2-MeImpG seems to be due to a template-directed process that facilitates incorporation of G_L residues. To investigate the possibility that this process is based on some

Table 1 Yield of oligo(G_D)s from the poly(C_D)-directed condensation of activated guanosine mononucleotides

System I		System II	
2-MeImpG	Oligo(G _D)	ImpG	Oligo(G _D)
0.025 M D-	74	0.01 M D-	30
0.05 M D-	75	0.02 M D-	19
0.05 M DL-	32	0.01 M DL-	2
0.10 M DL-	35	0.02 M DL-	2

Reaction conditions for systems I and II are given in the legends to Figs 1 and 2, respectively. Oligomer yields are the percentage of radioactive G_D residues incorporated into newly synthesized oligomers of tetramer length and longer. The values shown are the difference between template-directed oligomerization and oligomerization in template-free controls.

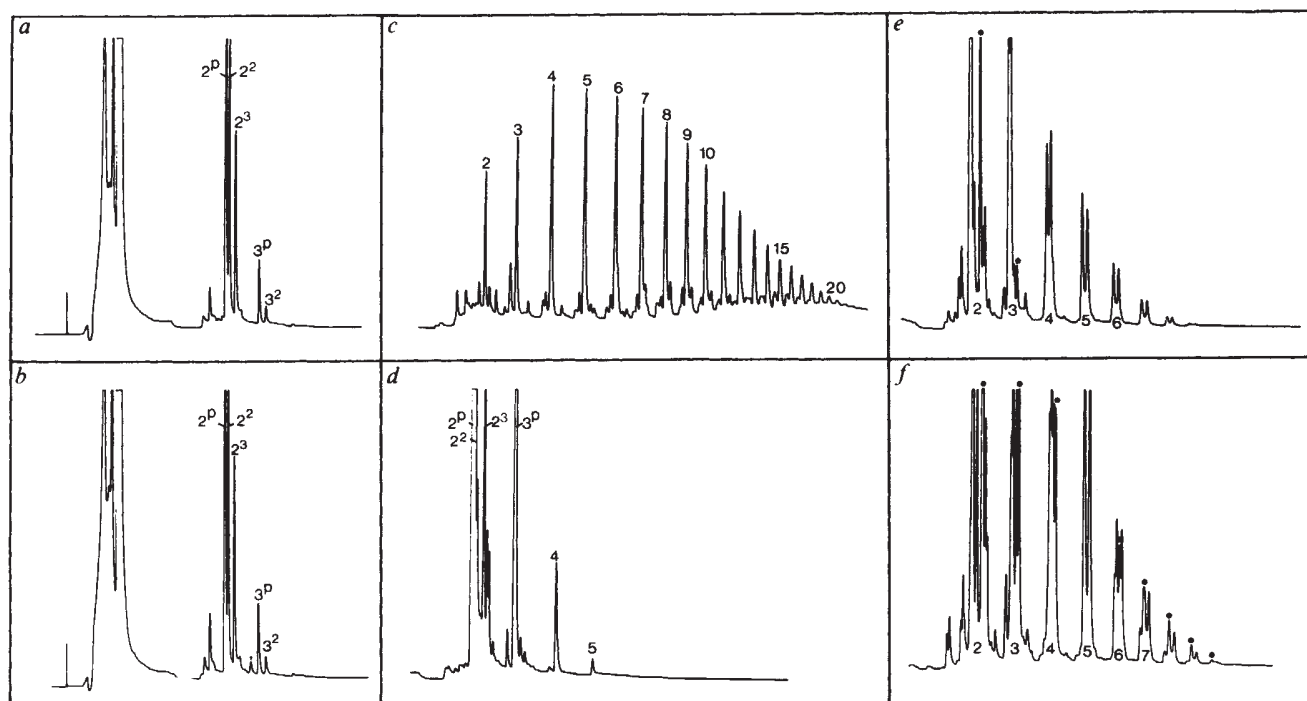


Fig. 2 HPLC elution profiles of 2-MeImpG condensation in the absence or presence of a poly(C_D) template. Reaction conditions for system I: 0 °C for 7 days; 1.0 M NaCl, 0.2 M $MgCl_2$, 0.2 M 2,6-lutidine-HCl (pH 8.0). *a*, No template, 0.05 M D-2-MeImpG; *b*, no template, 0.05 M L-2-MeImpG; *c*, 0.05 M poly(C_D), 0.05 M D-2-MeImpG; *d*, 0.05 M poly(C_D), 0.05 M L-2-MeImpG; *e*, 0.05 M poly(C_D), 0.05 M DL-2-MeImpG; *f*, 0.05 M poly(C_D), 0.1 M DL-2-MeImpG. The numbers labelling the peaks are the lengths of the corresponding oligo(G)s. 2^p is GppG, 2^2 is pG²pG, 2^3 is pG³pG, 3^p is Gp(pG)₂ and 3^2 is (pG)₂pG. Unreacted monomers are not shown in *c*–*f*. The peaks labelled with a dot in *e* and *f* correspond to (pG_D)_n. Conditions for HPLC: elution with a linear NaClO₄ gradient (pH 12, 0–0.04 M, 60 min) on an RPC-5 column; UV absorption monitored at 354 nm.

peculiarity of the 2-MeImpG reaction system, we performed a second series of experiments in different conditions.

System II involves the poly(C_D)-directed oligomerization of guanosine 5'-phosphorimidazole (ImpG) in the presence of Mg^{2+} and Zn^{2+} (ref. 4). Figure 3 shows HPLC elution profiles of the product distribution obtained from the various reactions. The results are similar to those obtained with system I. We carried out reactions using ¹⁴C-labelled D-ImpG and found that, in fact, G_L inhibition of oligo(G_D) formation is even more pronounced with system II (Table 1).

In poly(C_D)-directed reactions with a racemic mixture of 2-MeImpG, large amounts of G_L residues are incorporated as a 5'-terminal pyrophosphate. Increasing the concentration of monomers from 0.05 M to 0.1 M reduces the proportion of oligomers with a G_L pyrophosphate termination from 80% to 50%. Clearly, the L isomer forms pyrophosphate bonds more readily than the D isomer, and under competitive conditions, when the monomers are in excess, the D isomer interferes with L-2-MeImpG addition to the 5' terminus of template-bound oligo(G).

The rate of monomer addition to a template-bound oligomer is dependent on two factors: (1) the fractional occupancy by the monomer at the template site adjacent to the oligomer; and (2) the rate of condensation between template-bound monomer and an adjacent oligomer. 5'-Terminal G_D pyrophosphates are not formed in large amounts, and it is unlikely that D-2-MeImpG in solution could significantly alter the rate of condensation between a template-bound L monomer and an adjacent oligo(G). Thus, it seems that the D isomer interferes with G_L pyrophosphate formation by limiting the fractional occupancy of the L isomer. We conclude that under competitive conditions, D-2-MeImpG is attached to template sites adjacent to the 5' terminus of oligo(G) with higher affinity than L-2-MeImpG.

Addition of 2-MeImpG to the 3'-terminal hydroxyl of template-bound oligo(G) is not restricted by the presence of a terminal pyrophosphate at the 5' end of the oligomer⁸. Therefore, the inhibition of oligomerization by L-2-MeImpG must be

attributed to the presence of G_L residues at the 2'(3') end. Template-directed oligomerization of 2-MeImpG proceeds in the 5' → 3' direction⁵. D-2-MeImpG is incorporated more readily than L-2-MeImpG, but an incorporated G_L residue acts as a chain terminator that prevents further elongation. After extensive oligomerization, nearly all chains would have a G_L residue at the 2'(3') terminus. At that point the mean chain length provides an indication of the relative rates of addition of the two isomers.

Guanosine mononucleotide binds to poly(C) by forming a Watson-Crick base pair⁹. The orientation of the sugar-phosphate component of the monomer relative to the template is determined by the handedness of the ribose sugar (D versus L) and by the rotational conformation about the glycosidic bond (*syn* versus *anti*). The D isomer in the *anti* conformation and the L isomer in the *syn* conformation are oriented antiparallel to a poly(C_D) template, while the D isomer in the *syn* conformation and the L isomer in the *anti* conformation adopt a parallel orientation. This leads us to speculate that monomer addition to the 2'(3') terminus of a template-bound oligo(G_D) involves either the *anti* D or the *syn* L isomer, while addition to the 5' terminus involves either the *syn* D or the *anti* L isomer.

Guanosine mononucleotides in solution tend to adopt the *anti* conformation¹⁰; this may explain why the D monomer is more likely to be incorporated at the 2'(3') terminus and the L isomer is more likely to be incorporated at the 5' terminus (Fig. 4). Addition of D-2-MeImpG to the 2'(3') end is particularly favourable because the rate of condensation between monomer and acceptor has been specifically optimized¹¹. However, the structures of L-2-MeImpG in the *syn* conformation and D-2-MeImpG in the *anti* conformation are sufficiently similar to allow occasional misincorporation of G_L residues. Once a G_L residue is incorporated, the 2'(3') end of the oligomer is distorted, and further incorporation of either monomer is limited.

The chiral selectivity of the reaction is striking (see Figs 2c,d and 3a,b). Nonetheless, the inhibition of optically homogeneous template-directed oligomerization by monomers of the opposite

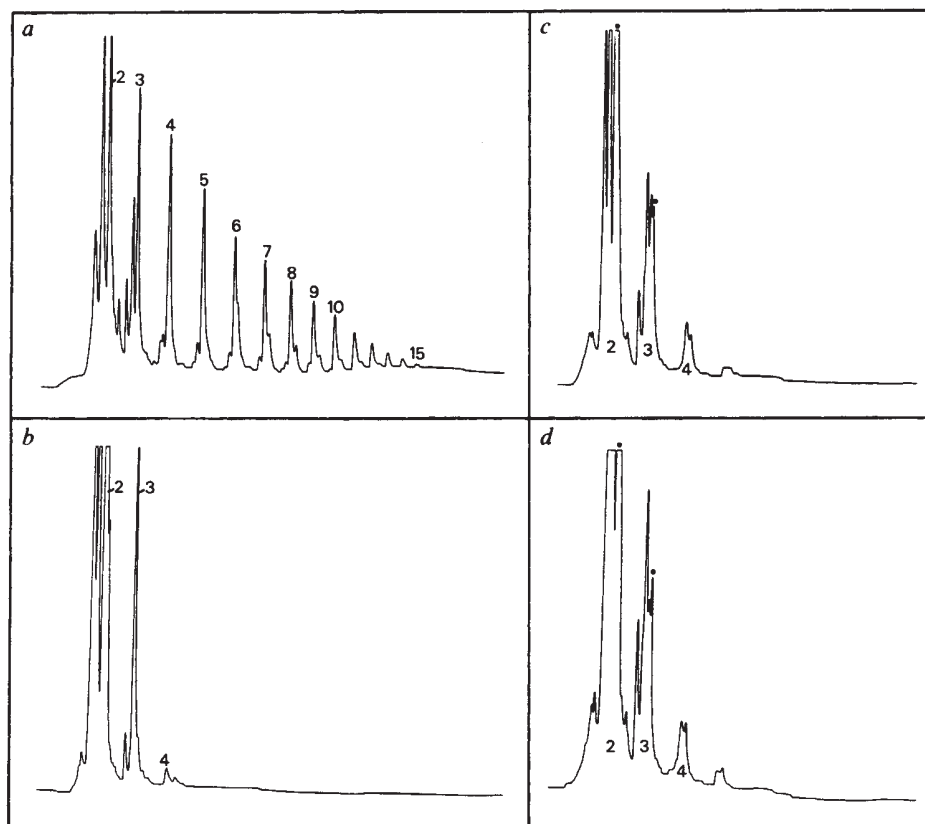
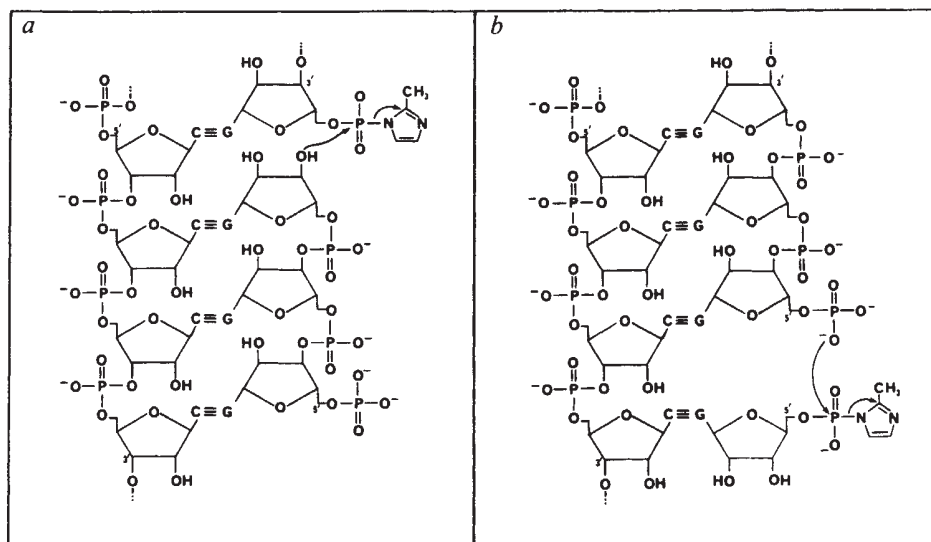


Fig. 3 HPLC profiles of ImpG condensation in the presence of a poly(C_D) template. Reaction conditions for system II: 0 °C for 7 days; 0.4 M $NaNO_3$, 0.5 M $Mg(NO_3)_2$, 0.2 M 2,6-lutidine- HNO_3 (pH 8.0), 0.02 M poly(C_D). *a*, 0.01 M D-ImpG; *b*, 0.01 M L-ImpG; *c*, 0.01 M DL-ImpG; *d*, 0.02 M DL-ImpG. The numbers are the peaks of the corresponding oligo(G)s. Unreacted monomers are not shown. The peaks labelled with dots in *c* and *d* correspond to $(pG_D)_n$. Conditions for HPLC: elution with a linear $NaClO_4$ gradient (pH 12, 0–0.04 M, 60 min) on an RPC-5 column; UV absorption monitored at 254 nm.

Fig. 4 Poly(C_D)-directed oligomerization of DL-2-MeImpG. *a*, Addition of *anti*-D-2-MeImpG to the 3' hydroxyl of template-bound oligo(G_D); *b*, addition of *anti*-L-2-MeImpG to the 5'-phosphate of template-bound oligo(G_D). It is assumed that the CG base pair formed by the incoming monomer has the same orientation as the previously formed CG base pairs. It then follows that the sugar-phosphate of an incoming *anti*-D monomer will be parallel to the sugar-phosphates of the oligo(G_D). On the other hand, the sugar-phosphate of an incoming *anti*-L monomer will be antiparallel to those of the oligo(G_D).



handedness (Figs 2*e,f* and 3*c,d*) should be recognized as an important problem for many theories of the origin of living systems. While it is conceivable that some prebiotic fractionation process allowed those chemicals necessary for the origin of life to become localized within a common microenvironment and excluded potential inhibitors, it is not possible that a comparable segregation of optical enantiomers could have been achieved in an achiral environment.

If the replication of polynucleotides was important at the earliest stages of life, there must have been a way to minimize

the effect of enantiomeric cross-inhibition. Our results with two different template-directed reaction systems suggest that there is some variability in the relative rates of incorporation of the two isomers. There may have been a prebiotic system in which the optical specificity of monomer incorporation was very high. If so, such a system would have enjoyed a considerable selective advantage in the prebiotic environment.

This work was supported by grants from the NIH and NASA. We thank A. R. Hill Jr for technical assistance and R. E. Brown for manuscript preparation.

Received 27 March; accepted 5 June 1984.

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Leonardo as he looked

Martin Kemp

Leonardo da Vinci's Elements of the Science of Man.

By Kenneth D. Keele.

Academic: 1984. Pp.385. \$90, £69.50.

IT is a pleasure to report that there is a great deal of Leonardo in Kenneth Keele's ingeniously planned and richly rewarding book. I do not just mean that it contains a wide range of familiar and unfamiliar quotations from Leonardo's notebooks — which it certainly does — but that its organization and approach is sensitively attuned to Leonardo's own patterns of thought.

Those patterns were very different from the ones prevailing in modern science, and it takes a positive act of will to view Leonardo's science on its own terms. The natural tendency in many of the pioneering studies was to assign plus marks for findings which were recognized as correct and to acclaim apparent examples of the experimental method which was seen as central to the scientific revolution. The practising scientists prominent amongst early interpreters were particularly predisposed to this approach. Although Keele is a retired physician, he is meticulous in attempting to examine Leonardo's thought historically in its own right, rather than extracting favoured ideas in order to force him into a proto-modern mould.

The principle behind the book's organization is broadly that outlined by Leonardo himself: "Arrange it so the book on the elements of machines with its practice shall precede the demonstration of the movement and force of man and other animals, and by means of these you will be able to prove all your propositions". Accordingly, Keele approaches his exposition of Leonardo's anatomical work, which occupies three-fifths of the volume, through an investigation of the physical principles underlying Leonardo's view of man, the "microcosm", in the context of the dynamics of the universal "macrocosm". This is entirely in keeping with Leonardo's desire to relate all his science to first principles, and, indeed, to obtain proofs in every field equivalent in certainty to those of mathematics.

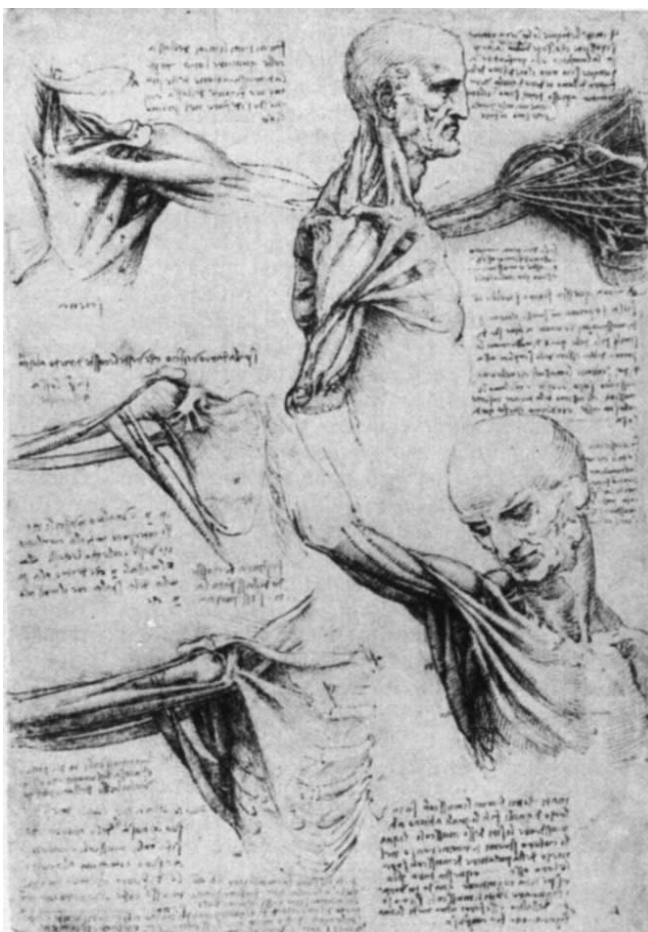
The problem at the heart of understanding his science is the relationship between Leonardo's much-vaunted emphasis

upon "experience" and his desire to explain even the most complex effects in terms of a limited set of predominantly Aristotelean causes. Leonardo's own statements, plucked from their historical context, were once enough to convince many that he was a great pioneer of the ex-

basis of the supposed causes and, typically in Leonardo's science, sketched in his notebooks. If the thought experiments "worked", the case was proven. Some of the models of the human eye, which look at first sight like records of actual apparatuses, are deductive variations of the joint bases of optical principles and the assumed structure of the refractory spheres of the eye. Equally, the optical "experiments" concerning the apparent size of objects on an intersecting plane, which Keele physically reconstructs, are almost certainly deductive variations based upon Piero della Francesca's treatise on perspective.

This raises the question of Leonardo's sources, the assessment of which is perhaps the weakest aspect of Keele's book. The author is an extremely persuasive interpreter of Leonardo's ideas, with a brilliant grasp of how the parts of Leonardo's science interlock with each other in patterns of astonishing complexity. But this exposition tends to float in a historical vacuum. Keele does show that Leonardo's procedures of dissection were directed to a significant degree by Mondino's *Anathomia*, but he does not acknowledge how far the underlying principles of his science — methodological, mathematical, dynamic, optical and so on — were those of the mediaeval Aristoteleans. Thus the "pyramidal law" which Keele rightly claims governs the performance (and diminution) of all the "powers of nature", is a facet of the mediaeval doctrine of the proportional laws of motion. And the ubiquitous effects of "percussion", again rightly emphasized by Keele, arise from the cause of "impetus", which is central to late mediaeval dynamics.

These kinds of technical terms from mediaeval science require careful handling, not least in translation. As the translator of the mammoth and massively expensive *Corpus of Anatomical Studies in the Collection of Her Majesty the Queen at Windsor Castle* (published in three volumes by Academic Press, price \$8,000!), Keele has already faced the problems of Leonardo's vocabulary in the most direct way. In the extensive quotations from the anatomical notes he is admirably sensitive to the need to avoid anachronistic rephrasing. And in his interpretative commentary he generally catches just the right flavour. The occasional lapses, as when he talks of the *imprensiva* as a "relay station" or the "circulation" of the humours, are therefore all the more marked. The non-



Leonardo da Vinci (1452–1519), as anatomist. These illustrations show the mechanics of the movement of the shoulder joint and are reproduced from *Leonardo da Vinci: Anatomical Drawings*, published by The Metropolitan Museum of Art, New York, and distributed in Britain by Blackwell Scientific. Price is \$35, £34.

perimental method. As we have come to understand mediaeval science more fully, so it has become clear that his approach in theory and in practice can be aligned with an earlier tradition of natural philosophy.

Leonardo did undertake experiments in the physical sense — some diagrams labelled *sperimentata* make this clear — but he did not differentiate the status of physical experiment from what we would call "thought experiments". In the latter cases, mental models of the effects are built on the

anatomical texts are less consistently handled, and he often relies upon the now-dated translations of Richter, with their late-nineteenth-century and sometimes mock-Elizabethan quality.

This raises the other main question concerning sources — Keele's own, and those the reader might use for further studies. For a book of its scholarly quality, it is astonishingly reluctant to acknowledge other literature on Leonardo. Some of the chapters are given a few scanty footnotes which do not always refer to the most central sources, and there is no consolidated bibliography. There is also a smattering of minor errors, particularly in the "Scientific Biography" which precedes the main chapters.

To end on a negative note, however, would be misleading. This is a thoroughly distinguished contribution to Leonardo studies. It can be taken as an authoritative exposition not only of the specific technical details of Leonardo's "microcosm" but also of the way these details profoundly express the character of his physical science as a whole. □

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Change in metallurgy

R.A. Cowley

Theory of Structural Transformations in Solids.

By Armen G. Khachaturyan.

Wiley: 1983. Pp.574. \$64.95, £57.45.

STRUCTURAL phase transformations in solids are studied by both physicists and metallurgists. In recent years considerable progress has been made by both groups, but it is alarming that there has been so little contact between them. The physicists have concentrated on the continuous phase transitions and largely, but not exclusively, on transitions in which the atoms move much smaller distances than the interatomic distances. During the 1960s they showed that the soft mode concepts developed by Cochran and Anderson were a fruitful way of describing these transitions, and more recently the effect of the critical fluctuations has been studied

using the concepts developed by Kadanoff, Wilson and Fisher.

It is, therefore, surprising to a physicist to find in Armen Khachaturyan's book no reference to the work of Cochran, Anderson, Kadanoff, Wilson or Fisher. The author admits that the Landau expansion of the free energy might not be mathematically correct at the transition point, but states that these problems only occur within a fraction of a degree outside of which the Landau expansion is valid. Such is the influence of Wilson's Nobel prizewinning work!

The subjects covered by Khachaturyan are those which have been studied by the metallurgist and which have been to a considerable extent ignored by the physicist. The transformations he considers are those occurring in metallic alloys and are mostly of the type which involve large atomic motions, there being detailed discussions of topics such as spinoidal decomposition and the shape of the inclusions and precipitates in various mixed phases.

Spinoidal decomposition occurs when an alloy of a certain composition is cooled to a temperature at which the composition is unstable against separation into a mixed phase of two different compositions. This separation clearly requires large atomic motions and so the kinetics is of importance. The shape of the inclusions of one phase in the other is then dependent on the strain developed at the surfaces of the inclusions, and a great deal of the book describes the work done by Khachaturyan and others on this difficult problem by assuming a static interface.

Much of the material stresses the application to particular alloy systems, and in this regard it is refreshing to read about real materials instead of the idealized models so often used by the physicist. But while the author has included considerable discussion of real materials, there are few experiments described which test the theory in detail. The description of the theory behind the experiments is also somewhat terse and is inaccurate at points — this is a pity because a better appreciation of the experimental possibilities might lead to a more comprehensive test of the theories.

This is a book which describes the metallurgist's approach to structural transformations in alloys. Physicists who wish to learn about the problems of mixed phases and spinoidal decomposition in metallic alloys will find it useful, as will its more natural audience of metallurgists. It is to be hoped that it will increase the number of people working on these systems who are aware of the importance of fluctuations in describing transitions, kinetics and interfaces, yet who are also able to handle the complications of real systems. □

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Old bottles, new wine

A. P. French

Understanding Relativity: Origin and Impact of a Scientific Revolution.

By Stanley Goldberg.

Birkhäuser: 1984. Pp.494. \$24.95. To be published in Britain on 20 September by Oxford University Press, £17.50.

STANLEY Goldberg has written an unusual book. Its subtitle, *Origin and Impact of a Scientific Revolution*, defines his theme — the study of the intellectual, social and cultural background against which a major change in our view of the physical world takes place. Relativity *per se* is not the issue, but the special theory of relativity provides a particularly illuminating case study because its history belongs to twentieth-century physics, its development is incomparably well documented and it is, in the strictest sense, a very simple theory, the fundamental postulates of which were first spelled out by Einstein in just two easily understood sentences. Yet the acceptance of Einstein's theory, following its publication in 1905, was a prolonged and tortuous affair which, in Goldberg's view, is still not complete, even within the scientific community. Why is this? It is Goldberg's aim to explore the question in a way that is accessible to any interested reader; he has relegated almost all mathematical material to a series of appendices.

Goldberg begins by contrasting mathematical and scientific forms of knowledge. He emphasizes that, unlike mathematics, science is not a tightly contained logical system. Different theories can be devised to account for any given set of observations, and criteria other than logic are involved in preferring one theory over another. Many scientists follow Einstein in attaching great importance to a theory's economy and beauty, but entrenched attitudes and assumptions also play a role, as the history of relativity itself makes abundantly clear.

To pave the way for the discussion of Einstein's theory, Goldberg describes the development and subsequent decline of the mechanical description of the Universe. The luminiferous ether was one important product of this description, but with bizarre properties — so tenuous as to offer negligible resistance to the planets, yet so astoundingly elastic that its vibrations, in the form of light, travelled at a million times the speed of sound. Then, in the mid-nineteenth century, came the spectacular flowering of electromagnetic theory, and a swing to the belief that electricity was the key to the structure of matter and the nature of light. Maxwell's equations, yielding the numerical value of the speed of light, were a seemingly decisive clue to the properties of the ether. Nobody doubted the existence of this medium, although it refused to reveal itself in the face of tests

New in paperback

• *The Surface of Mars* by Michael H. Carr, which first appeared in 1982. The book is in large-format to accommodate the great number of photographs, and is published by Yale University Press. Price is \$24.95, £19.95. For review see *Nature* 299, 761; 1982.

• *Principles of Stellar Evolution and Nucleosynthesis*, Donald D. Clayton's textbook first published by McGraw-Hill in 1968. The book contains a new preface by the author, and has been re-issued by The University of Chicago Press. Price is \$15, £12.75.

designed to detect our motion through it. Accounting for these failures through such mechanisms as the Lorentz contraction entailed a great elaboration of the electrical theory of matter during the years 1890–1905.

Into the midst of this complexity, Einstein brought his theory with its two basic postulates: relativity is a universal condition (no preferred ether frame) and the speed of light is a universal invariant. From this simple beginning, all else followed — length contraction, time dilation, mass varying with speed and so on. Surely this brilliant solution should have been immediately acclaimed and universally accepted. But what actually happened? For a couple of years, except in Germany, it was almost totally ignored; thereafter it was widely resisted. In discussing why that was so, Goldberg considers the reception of the theory in four different cultures — German, French, British and American. In a brief review it is hard to do justice to Goldberg's analysis, but he suggests that Germany's academic tradition, and its strongly interlinked academic structure, encouraged active debate. By contrast, any French reaction may have been stifled by the extreme conservatism of that country's educational system — coupled with a lack of enthusiasm on the part of Poincaré, who himself came so close to inventing relativity. In Britain the love of mechanical models, and a suspicion of abstractions, made many distinguished physicists reluctant to abandon the ether. And in the United States the main obstacle was the conviction "that acceptable theories had to conform to acceptable notions of common sense", a precept which Einstein's theory clearly violated! Goldberg's identification of such national and cultural differences is interesting, although he himself dismisses any thought of a "party line" in such matters.

The last part of the book discusses the assimilation of relativity in the United States after 1912. Much of this deals with the question of whether the invariance of the speed of light is amenable to experimental test or is, as Einstein presented it, an untestable postulate. Goldberg concludes that, even today, many American physicists take the former view (which makes no difference, of course, to their acceptance and use of Einstein's equations).

All in all this is a rewarding book, whether or not one agrees with its particular theses and interpretations. For anyone who is not a scientist, it will provide a healthy (and intended) antidote to the view that the progress of science is impersonal and inevitable. And for the professional physicist, it offers a wealth of background to the history of special relativity as such. □

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Sulphur in movement

C.E. Rees

The Global Biogeochemical Sulphur Cycle.

Edited by M.V. Ivanov and J.R. Freney.
Wiley: 1984. Pp. 470. \$79.95, £42.50.

SULPHUR is only a minor constituent ($\sim 260 \mu\text{g g}^{-1}$) of the Earth's crust, much of the original complement of the element either being lost to space, along with other volatiles, during the accretion of the planet, or transported to the core during early differentiation. Nonetheless sulphur is important both geochemically and biologically. It is essential to life, because of its presence in the amino acids cysteine, cystine and methionine, and is crucial to modern man because of its use in the agricultural and chemical industries. Further, it is an unwanted constituent of fossil fuels and is being released into the environment at an increasing rate with consequences which are receiving increasing publicity.

The chemistry of crustal sulphur is a complex matter, both because of the different oxidation states in which the element can exist and because of its involvement in a wide variety of biological and geological processes. The major crustal reservoirs are sulphate dissolved in the oceans, sulphate stored as evaporitic minerals and reduced forms of sulphur in sediments. Cycling occurs through these reservoirs on a rather long time-scale ($\sim 10^7$ yr) by three main processes: bacterial reduction of ocean sulphate to form sedimentary sulphides; the formation of evaporitic sulphate minerals; and the return of sulphate to the oceans by erosional processes. Other small, though still important, reservoirs of sulphur include fresh water, the atmosphere and the biosphere (of which the sulphate-reducing bacteria form only a small and rather odd part).

The Global Biogeochemical Sulphur Cycle is an attempt, and in my view a highly successful one, to summarize what is known about the geological and biological reservoirs of crustal sulphur and how the element is cycled between them. The natural (or pre-industrial) sulphur cycle receives most attention, but where possible information is given concerning the perturbations of the natural cycle by the activities of man — sulphur is now being added to the oceans at perhaps twice the pre-industrial rate, and present-day emissions of sulphur to the atmosphere from the combustion of fossil fuels and ore smelting probably exceed natural emissions by a factor of two to three.

The book begins with a brief introductory essay — "Principal Reactions of the Global Biogeochemical Cycle of Sulphur" — after which individual chapters cover the sulphur cycle in the lithosphere, in soil, in the atmosphere, in continental reservoirs

and in oceans. There is a final chapter which summarizes the information available on the major fluxes of sulphur within and between the various reservoirs. Of the eleven contributors to the book, nine are Soviet scientists. This is not a weakness because the coverage of the subject is clearly global in scope; indeed, it is valuable to have a summary in English of the wide Soviet literature on the subject.

Preparation of material for the book was essentially complete by 1979 when an international workshop was held in the USSR to offer comments on draft versions of the various chapters. Accordingly the literature cited is largely that published before 1980. The authors, editors and, where appropriate, translators, as well as the participants in the workshop, are to be congratulated on their production of a synthesis of data available up to that date which is exhaustive in its detail but well organized and clear in style. □

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Quality for quantity?

J.R. Ravetz

The Citation Process. By Blaise Cronin.
Taylor Graham, 500 Chesham House,
150 Regent Street, London W1R 5FA:
1984. Pp. 103. Pbk £10.

THIS elegant little study takes its origin from a paradoxical aspect of the social activity of science — that the system of citation of previous research, performing several essential functions for that activity, has only very recently been appreciated as at all problematic. It is scarcely 15 years since critical analysis of the citation process began; and as the author shows, it is still very marginal, divided and incapable of offering much practical advice.

In principle, citations enable science to be truly "public knowledge" by providing techniques for checking on the sources of materials used in a paper but produced elsewhere. And they are the main regular means for the validation of the intellectual property embodied in a paper; the users of that property "pay" through the credit given to its author in the cited reference. In the idealistic image of science codified in the "four norms" of Robert K. Merton, there was no occasion for either of these functions to be other than straightforward. But with the rise of a critical, even demystifying sociology of science, it was discovered that the practice of citation was strongly subject to personal interpretation and also to political manipulation. When the *Science Citation Index* became available, providing what seemed to be an objective measure of quality of papers and of researchers, and was invoked in legal disputes over promotion and status, the quality-

assessment of this tool of quality assessment took on real practical urgency.

Blaise Cronin narrates the interaction between the development of the new sociology of science and the increasing sophistication in the criticism of citations. His basic insight is that citation is an intensely personal process (a "subtle craft skill" for which there is scarcely any formal training) which has a product that is public, and moreover of great importance in the government of science. He reviews the leading positions on the sociological side (those of myself, I.I. Mitroff and the "social construction" or "lab-life" school), and brings us up to date on the attempts to bring some order into the citation process. But it is still an open question whether any scheme that is sufficiently simple to be effective as a guide to practice can encompass both the complexities of the cognitive aspects of that process and the subtleties of the motivational side. And in the last resort, the communities of working scientists still show remarkably little interest in the whole problem.

Cronin's analysis suffers, in my opinion, from its failure to take note of the policy issues which have shaped the study of citations during its brief history. The reliance on citations was part of the "science of science" movement led by Bernal's disciple D.J. Price, and now carried on as "scientometrics". The challenge of the *Science Citation Index* led to the critical and ultimately self-critical discussions of the "science indicators" movement. It is significant that more recent consideration of the assessment of quality in science has moved from the original quantitative techniques to deeper problems of the ultimately political processes of the setting of dominant criteria of quality.

Within its limitations, this study does offer a condensed synoptic survey, and also many useful bits of information. For example, the *Science Citation Index* works from a mere 2,000 journals, thus providing a practical solution to the problem of the "information explosion" which seemed so urgent in the 1960s. By the fiat of its advisors, the overwhelming majority of journals and of published papers are deemed as failures, not worth noting in this standard quality-index.

To conclude with one small personal grievance: Cronin says nothing about the form of citations, and seems to consider that as unproblematic. Thus he himself automatically cites materials from a particular edition at hand, even though this may radically distort the historical process. R.K. Merton's fundamental work is dated to 1973 (some 30 years late) though the author confusingly refers to a "subsequent" publication of 1957. But this small defect in citation technique should not prevent the book's being cited, or even being read. □

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Parasites in the lab

F. E. G. Cox

Genes and Antigens of Parasites: A Laboratory Manual, 2nd Edn.

Edited by C.M. Morel.

Dept Biochemistry and Molecular Biology, Instituto Oswaldo Cruz, Rio de Janeiro: 1984. Pp.586. Available from A.O. Lucas, SPRTTD, WHO, 1211 Geneva 27, Switzerland. No charge.

THE intrusion of molecular biology into parasitology has necessitated the production of a laboratory manual, to serve both as a book of recipes for laboratory investigations and as a source of information for parasitologists bewildered by terms such as genomic libraries, Western blotting and zymodemes. Here it is.

This cook-book is based on the laboratory manual used during a five-week course on genes and antigens of parasites, held at the Oswaldo Cruz Institute in Brazil at the end of 1983. The published version, revised and enlarged, is spirally bound, in traditional cook-book fashion, and contains 34 chapters organized into five sections — general techniques, character-

ization of parasites, recombinant DNA, surface antigens and hybridoma technology. The chapters are by scientists experienced in their fields and each is set out with step-wise instructions and elaborate technical details. The book is designed to be informative and in that it succeeds, although suffering a little from the lack of a proper index.

The emphasis is largely on Chagas' disease and leishmaniasis; however the general principles apply to parasites that cause other diseases and the 628 references will guide the investigator into the literature of the subject as a whole. No cook-book can be completely satisfactory, and the techniques may have to be modified, but there is plenty of space for the pencilled amendments that quickly characterize a book of this kind. *Genes and Antigens of Parasites* will be invaluable not only to research workers but also to teachers planning up-to-date practicals in parasitology. Every active parasitologist should have a copy, and should also respond to the editor's request for comments which will ensure the publication of a third and even better edition. □

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A plague behind us

Keith Dumbell

Princes and Peasants: Smallpox in History.

By Donald R. Hopkins.

University of Chicago Press: 1984. Pp.380. \$28.75, £21.25.

THE eradication of smallpox has been an undersung triumph. Europe and North America have, for a generation, known smallpox only as an occasional visitant, following the arrival of infected persons from endemic areas. Despite some inevitable panic, each incident has been effectively contained and eliminated.

How different was the situation a couple of generations earlier when smallpox was a fact of life. And how different it was in those countries where the disease remained endemic and where, even in 1967, smallpox killed about two million of the 10–15 million people now believed to have been infected in that year.

As part of the epitaph of smallpox, it was necessary for someone to remind us of the magnitude of the scourge that has gone. Donald Hopkins set out to tell the story of smallpox, but what particularly fascinates him is the role that the disease has played — whimsical, unpredictable and often devastating — in the daily life of mankind and the course of history.

Some of the episodes are fairly well known, but nowhere else that I know is

there to be found such a compact account of smallpox in all five continents and of its impact on the history of each. Smallpox neutralized armies and removed princes at politically critical times. It played a continual part in determining the events that political and social history record. Hopkins follows this theme with vigour and includes, for good measure, some near misses such as the delivery of the Gettysburg address the very evening before President Lincoln himself became a temporary victim of smallpox. By necessity the author casts only a glance down some intriguing avenues, but the extensive reference list will help readers who wish to explore them in greater detail.

While Hopkins shows us the potency of smallpox virus as mankind's enemy, he does not stress how relatively puny was the David that overthrew this Goliath. *Princes and Peasants* is a prologue to the story of eradication, a tribute to the disease that has been beaten. There is no forecast of other plagues to conquer, no comment on other achievements within the reach of a similar international co-operation. Indeed this is not a reflective book; it is a simple narration of events, written with obvious enthusiasm. I found it a sobering and broadening experience to be exposed to Hopkins's story — there will be few people who would not benefit from reading it. □

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